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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002950217 for a patent by PRANA BIOTECHNOLOGY LIMITED as filed on 16 July 2002.

WITNESS my hand this
Twenty-third day of July 2003

J. Billingsley

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s):

PRANA BIOTECHNOLOGY LIMITED

Invention Title:

8-HYDROXY QUINOLINE DERIVATIVES

The invention is described in the following statement:

8-HYDROXY QUINOLINE DERIVATIVES

The present invention relates to 8-hydroxy quinoline derivatives, processes for their preparation and their use as pharmaceutical or veterinary agents, in particular for the treatment of neurological conditions, more specifically neurodegenerative conditions such as Alzheimer's disease.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

The life span is thought to be biologically fixed for each species, and the length of the human life span is uncertain, but may be up to 120 years. Since life expectancy has risen significantly in this century, the elderly are an increasing segment of our population, and their health care needs will continue to grow for decades.

Although normal aging is characterized by modest reductions in the mass and volume of the human brain, which may be due to the atrophy and/or death of brain cells, these changes are far more profound in the brains of patients who succumb to a neurodgenerative condition. Most of these conditions are sporadic (i.e., not due to genetic mutations) and of unknown cause, but hundreds of different mutations in many genes have been shown to cause familial (inherited) variants of several neurodegenerative conditions. Many of the dozen or more genes that harbor these mutations were discovered in the quest to determine the genetic basis of neurodegenerative conditions just in the last ten years. Neurodegenerative conditions evolve gradually after a long period of normal brain function, due to progressive degeneration (i.e., nerve cell dysfunction and death) of specific brain regions. Since symptomatic expression of disease occurs when nerve cell loss exceeds a "threshold" for the continuing function (e.g., memory, movement) performed by the affected brain region, the actual onset of brain degeneration may precede clinical expression by many years.

Intellectual and higher integrative cognitive faculties become progressively impaired and interfere with activities of daily living in neurological conditions resulting in dementia. The precise prevalence of dementia in the elderly population is unknown, but may be 15% of people over 65 years old with 5% severely and 10% mildly to moderately demented. The prevalence of severe dementia increases from 1% at 65 years to 45% at 85 years. There are

many causes of dementia, but Alzheimer's Disease (AD) accounts for 50% of demented patients over 65 years of age.

AD is a primary degenerative disease of the brain. It is characterized by progressive decline of cognitive functions such as memory, thinking, comprehension, calculation, language, learning capacity and judgement. Dementia is diagnosed when these declines are sufficient to impair personal activities of daily living. AD shows an insidious onset with slow deterioration. This disease needs to be clearly differentiated from age-related normal decline of cognitive functions. The normal decline is much less, much more gradual and leads to milder disabilities. The onset of AD is usually after 65 years of age, although earlier onset is not uncommon. As age advances, the incidence increases rapidly (it roughly doubles every 5 years). This has obvious implications for the total number of individuals living with this disorder as life expectancy increases in the population.

The aetiology of dementia of AD is unclear. There is considerable evidence of a heritable predisposition for some forms of AD (reviewed in St George-Hyslop, 2000), and the expression of certain isoforms of ApoE has also been linked to a higher risk of AD (Corder et al, 1993; Czech et al 1994). The toxic accumulation of aluminium has been suggested as a causative agent in AD, although this hypothesis has now been largely superseded. The brains of AD patients display abnormal deposits which include β-amyloid protein (Aβ).

Aβ is known to be present in the brains of individuals with certain neurodegenerative diseases, but it is not known whether it is symptomatic of an underlying disease process, or is actually involved in the aetiology of the disease. For example, some authors believe that the Aβ deposits may be indicative of a normal brain defence mechanism, in which the brain attempts to sequester the Aβ; such deposits can be present in the brains of normal individuals. There is a mutation of *tau* protein in which neurofibrillary tangles, but no amyloid plaques are present in the brain; this condition is known as tauopathy.

One proposed approach to AD therapy is to inhibit production of Aβ in the brain. Proteolytic cleavage of APP by BACE1 and γ-secretase generates the full-length Aβ, which is then released from cells (Nunan and Small, 2000). Therefore inhibitors of either BACE1 or g-secretase may be of therapeutic value. Alternatively, a number of studies have shown that cholesterol can influence Aβ release (Simons et al., 1998; Hartmann, 2001; Fassbender et al., 2001; Frears et al., 1999; Friedhoff et al., 2001). Therefore inhibitors of cholesterol biosynthesis, such as statins, may also be of therapeutic value. One advantage of statins is that they have relatively low toxicities, and their mode of action is much better understood than many other compounds currently being investigated as therapeutic agents for AD. However, there is some disagreement in the art as to the value of lowering cholesterol levels, and some workers consider that cholesterol is actually beneficial. For example, Ji et al, (2002) have suggested that the binding of Aβ to cholesterol might prevent Aβ toxicity by inhibiting its

oligomerization.

Aβ can bind to lipids (Curtain et al., 2001; Valdez-Gonzalez et al., 2001), including gangliosides (Ariga et al., 2001).

It has been suggested that Aβ can bind to membrane lipids, and this interaction may be toxic for cells (Hertel et al., 1997). However, few studies have attempted to correlate the degree of lipid binding by Aβ with its toxicity.

It is also known that acetylcholinesterase (AChE) colocalises with Aβ in the amyloid deposits which are found in the brains of Alzheimer's disease patients, and that AChE accelerates amyloid formation, both from wild-type Aβ and from a mutant Aβ peptide which alone is able to produce few amyloid-like fibres.

In an alternative approach, it has been proposed that by unravelling the proteolytic processing of the amyloid precursor protein (APP), which generates the Aβ amyloid monomer, a number of possible therapeutic targets may be possible (Shearman et al., 2000; Sinha et al., 1999);], and this approach is in an early stage of clinical development. Attempts to promote the clearance of Aβ from the brain through immunization with Aβ, while efficacious in a transgenic mouse model for AD (Schenk et al 1999), have been found to have significant adverse effects (Brower, 2002).

It has also been suggested that deposition of amyloid-like fibrils may also be important in other neurodegenerative diseases. These include Parkinson's disease, dementia with Lewy body formation, multiple system atrophy, Hallerboden-Spatz disease, and diffuse Lewy body disease.

One of the competing theories of the aetiology of AD is that the causative step(s)lies within the pathway of the intracerebral biogenesis and accumulation of the Aβ amyloid protein (see recent reviews by Selkoe, 2001; Beyreuther et al., 2001; Bush, 2001). However, to date no drugs or agents which target this pathway have been demonstrated to have a lasting effect on modifying the clinical expression of the disease or in preventing or ameliorating the decline in cognitive function associated with neurodegenerative disorders, including Alzheimer's disease.

A further hypothesis is that AD is caused by the toxic accumulation of Aβ amyloid, due in part to excess binding of copper and zinc, metal ions which are abundant in the regions most affected. Moreover, it has been suggested that when Zn^{2+} and Cu^{2+} ions interact with Aβ, aggregation of Aβ into fibrils and plaques occurs (Atwood et al., 1998; confirmed by recent data from animals deficient in synaptic Zn^{2+} (Lee et al., 2002). It has also been suggested that redox-active Cu^{2+}-Aβ interactions can generate H_2O_2 from O_2 (Huang et al., 1999). Both Cu^{2+} and Zn^{2+} have been shown to affect Aβ-lipid membrane interactions (Curtain et al., 2001).

A method of treatment of AD using iodochlorohydroxyquinoline an antibiotic [also known as clioquinol (CQ)], is disclosed and claimed in US patent Nos. 5,994,323 and

6,001,852 by P.N. Geromylatos S.A. and in US patent application No. 09/972,913 by Bush *et al.* CQ was withdrawn as an antibiotic in 1970, because of its association with an uncommon neurological syndrome, subacute myelo-optic neuropathy (SMON), which was observed only in Japan in the 1960s, in patients thought to have received the drug over long periods and probably at doses higher than those recommended at the time (Shiraki, 1975). However, recent evidence suggests that SMON was caused by an overuse-related vitamin B12 deficiency in an exceptionally vulnerable population, and therefore could be rehabilitated for study in a clinical setting (Yassin et al., 2000; Bush and Masters, 2001).

However, no *in vivo* results in animal models or in humans are provided in the Geromylatos and Bush patents. US 5,994,323 discloses a composition comprising and Vitamin B12, and its use for the treatment of "diseases or disorders responsive to CQ administration while inhibiting detrimental side effects" of CQ. These diseases include AD. US 6,001,852 discloses a method of treatment of AD using CQ, preferably together with Vitamin B12. Both US 5,994,323 and US 6,001,852 suggest a dosage of 10-750 mg per day; US 5,994,323 recommends that if treatment is over a long period CQ should be given intermittently, for up to 3 weeks at a time followed by a "wash-out" period of 1-4 weeks.

In US application No.09/972,913 CQ is exclusively referred to in terms of its ability to disaggregate Aβ deposits. No other mechanism of neurotoxicity is discussed. PCT/US99/05291 by General Hospital Corporation discloses the use of CQ in combination with specific copper and zinc chelators to promote dissolution of amyloid plaques and inhibition of amyloid plaque formation and/or the production of ROS by Aβ.

US 6,001,852 also suggests that a composition comprising CQ and Vitamin B12 could be used in the treatment of Parkinson's disease; however, in this context it is suggested that CQ acts primarily via clearing iron from the substantia nigra.

The efficacy of CQ in the treatment of AD rests upon its ability to enter the CNS and then sequester the transition metals Cu, Zn and Fe from various Aβ entities thereby reducing Aβ toxicity and liberating it for clearance. The effectiveness of CQ is restricted by its poor aqueous solubility which limits its oral bioavailability. CQ is also known to undergo considerable conjugative metabolism and has a history of toxicity as discussed above. The fact that CQ is a bidentate metal ligand makes necessary the commitment of at least two molecules for every metal ion captured.

We have now developed 8-hydroxy quinoline derivatives which are more efficacious than CQ through the collective optimization of one or more of the following properties:

(a) metal chelation;

(b) aqueous solubility;

(c) improved cell toxicity;

(d) amyloid dispersion properties;

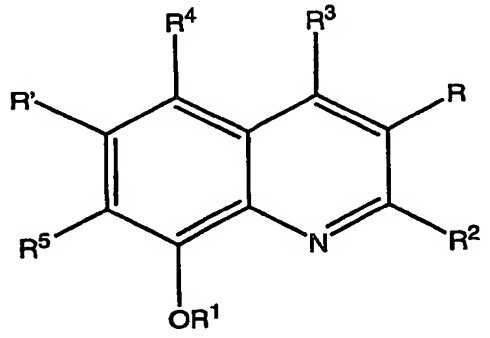
(e) membrane permeability appropriate for CNS penetration; and

(f) metabolic stability.

These derivatives include examples of therapeutics which are concentrated in the CNS through active transport, contain antioxidant activity in addition to their metal chelation properties which in some cases leads to enhanced metal chelation properties and demonstrate a prodrug strategy which masks the 8-hydroxy moiety to favour CNS penetration and make use of the known esterase activity which resides on the inner surface of the blood brain barrier (BBB).

SUMMARY OF THE INVENTION

According to the present invention there is provided a method for the treatment and/or prophylaxis of a neurological condition which comprises the administration of an effective amount of a compound of formula I:



II

in which

R^1 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted acyl, optionally substituted aryl, optionally substituted heterocyclyl, an antioxidant or a targeting moiety;

R^2 is H; optionally substituted alkyl; optionally substituted alkenyl; optionally substituted aryl; optionally substituted heterocyclyl; optionally substituted alkoxy; an antioxidant; a targeting moiety; COR^6 or CSR^6 in which R^6 is H, optionally substituted alkyl, optionally substituted alkenyl, hydroxy, optionally substituted aryl, optionally substituted heterocyclyl, an antioxidant, a targeting moiety, OR^7, SR^7 or NR^7R^8 in which R^7 and R^8 are either the same or different and selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted aryl or optionally substituted heterocyclyl; CN; $CH_2NR^9R^{10}$, $HCNOR^9$ or $HCNNR^9R^{10}$ in which R^9 and R^{10} are either the same or different and selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted aryl or optionally substituted heterocyclyl; OR^{11}, SR^{11} or $NR^{11}R^{12}$ in which R^{11} and

R^{12} are either the same or different and selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted aryl or optionally substituted heterocyclyl or together form optionally substituted heterocyclyl; or $SO_2NR^{13}R^{14}$ in which R^{13} and R^{14} are either the same or different and selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted aryl or optionally substituted heterocyclyl; and

R^3, R^4, R^5, R and R' are either the same or different and selected from H, optionally substituted alkyl, optionally substituted alkenyl optionally substituted alkoxy, optionally susbsituted acyl, hydroxy, alkylamino, alkylthio, alkylsulphonyl, alkylsulphinyl, halo, SO_3H, amine, optionally substituted aryl, optionally substituted heterocyclyl, an antioxidant or a targeting moiety,

with the proviso that when R^1 to R^3, R and R' are H, then R^4 is not Cl and R^5 is not I,

salts, hydrates, solvates, derivatives, pro-drugs, tautomers and/or isomers thereof to a subject in need thereof.

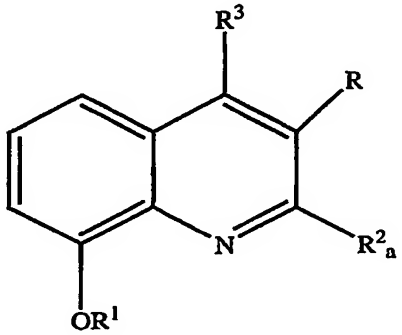
The invention also provides use of the compound of formula I in the manufacture of a medicament for the treatment and/or propylaxis of a neurological condition.

The invention further provides use of the compound of formula I for the treatment and/or prophylaxis of a neurological condition.

The invention still further provides use of the compound of formula I as a pharmaceutical, preferably a neurotherapeutic or neuroprotective agent, more preferably an antiamyloidogenic agent. Preferably, the neurological condition is a neurodengerative condition, more preferably neurodengenerative amyloidosis such as Alzheimer's disease.

Preferred compounds of formula I are as follows:

(i) Formula Ia



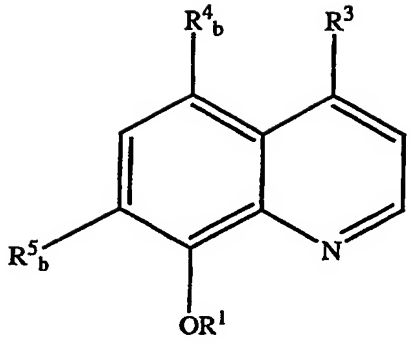
in which:

R, R^1 and R^3 are as defined in formula I above; and

$R^2{}_a$ is H; optionally substituted C_{1-6} alkyl; optionally substituted C_{1-6} alkenyl;

optionally substituted aryl; optionally substituted heterocyclyl; an antioxidant; a targeting moiety; COR^6_a or CSR^6_a in which R^6_a is H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, hydroxy, optionally substituted aryl, optionally substituted heterocyclyl or OR^7_a, SR^7_a or $NR^7_aR^8_a$ in which R^7_a and R^8_a are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted hetercyclyl; CN; $CH_2NR^9_aR^{10}_a$, $HCNOR^9_a$ or $HCNNR^9_aR^{10}$ in which R^9_a and R^{10}_a are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted heterocyclyl; OR^{11}_a, SR^{11}_a or $NR^{11}_aR^{12}_a$ in which R^{11}_a and R^{12}_a are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted heterocyclyl or together form optionally substituted heterocyclyl; or $SO_2NR^{13}_aR^{14}_a$ in which R^{13}_a and R^{14}_a are either the same or different and selected from H or optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted heterocyclyl.

(ii) Formula Ib



Ib

in which:

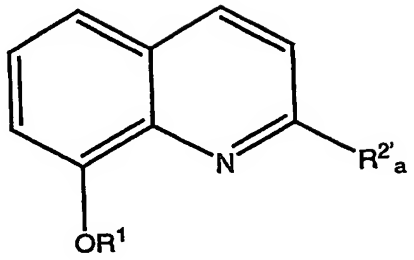
R^1 and R^3 are as defined in formula I above;

R^4_b and R^5_b are either the same or different and selected from H; optionally substituted C_{1-6} alkyl; optionally substituted C_{2-6} alkenyl; halo; an antioxidant; a targeting moiety, SO_3H; $SO_2NR^{13}_aR^{14}_a$ in which R^{13}_a and R^{14}_a are as defined in formula Ia above; or OR^{15}_b, SR^{15}_b or $NR^{15}_bR^{16}_b$ in which R^{15}_b and R^{16}_b are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{1-6} acyl, optionally substituted aryl or optionally substituted heterocyclyl,

with the proviso that when R^1 and R^3 are H, then R^4_b is not Cl and R^5_b is not I.

Preferred compounds of formula Ia are as follows:

(iii) Formula IIa



IIa

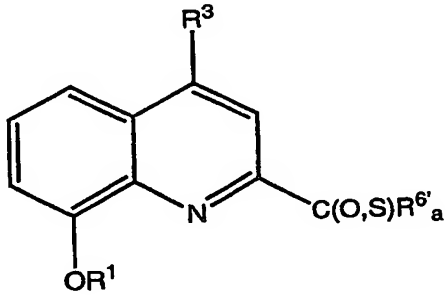
in which:

R^1 is as defined in formula I above; and

$R^{2'}_a$ is optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted heterocyclyl.

Formula IIa may represent compounds in which an antioxidant moiety is attached to the C2 position of the 8-hydroxyquinoline in such a way that exposure to a prooxidative environment, that is, hydroxy radicals, will result in a molecule with enhanced metal chelation properties.

(iv) Formula IIIa



IIIa

in which:

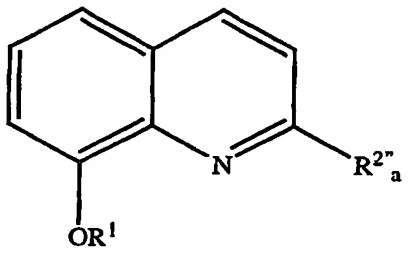
R^1 and R^3 are as defined in formula I above; and

$R^{6'}_a$ is optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, hydroxy, $OR^{7'}_a$, $SR^{7'}_a$, $N_2R^{7'}_aR^{8'}_a$, or $NR^{7'}_aR^{8'}_a$ in which $R^{7'}_a$ and $R^{8'}_a$ are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted aryl or optionally substituted heterocyclyl.

Formula IIIa represents compounds in which a hydrophilic amide moiety is

attached to the C2 position of the 8-hydroxyquinoline so as to generally enhance solubility while maintaining membrane permeability. Compounds of formula IIIa also show enhanced metal chelation properties.

(v) Formula IV



IVa

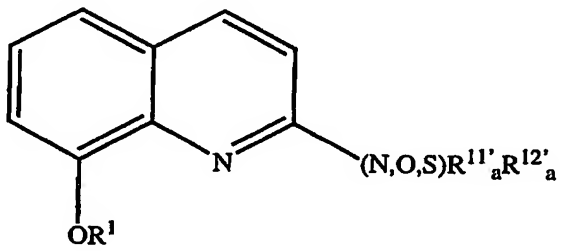
in which:

R^1 is as defined in formula I above; and

$R^{2"}{}_a$ is CN; $CH_2NR^{9'}{}_aR^{10'}{}_a$, $HCNOR^{9'}{}_a$ or $HCNNR^{9'}{}_aR^{10'}{}_a$ in which $R^{9'}{}_a$ and $R^{10'}{}_a$ are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted alkenyl, optionally substituted aryl or optionally substituted heterocyclyl.

Formula IVa represents compounds which have improved metal chelation and optimised activity in the panel of assays described hereinafter.

(vi) Formula Va



Va

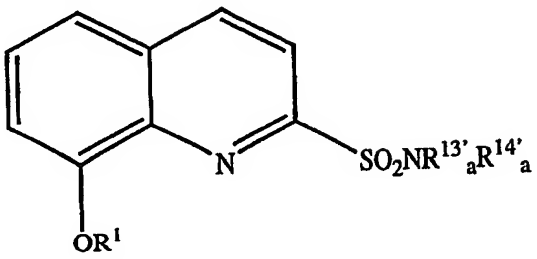
in which:

R^1 is as defined in formula I above; and

$R^{11'}{}_a$ and $R^{12'}{}_a$ are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl and

optionally substituted heterocyclyl or together form optionally substituted heterocyclyl.

(vii) Formula VIa



VIa

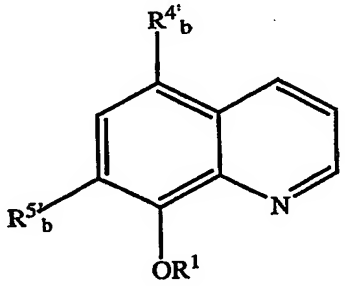
in which:

R^1 is as defined in formula I above; and

$R^{13}{}_a{}'$ and $R^{14}{}_a{}'$ are either the same or different and selected from H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted aryl or optionally substituted heterocyclyl.

Preferred compounds of formula Ib are as follows:

(viii) Formula IIb



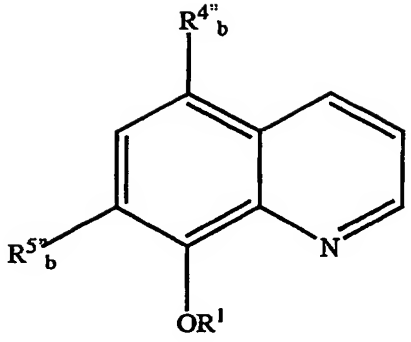
IIb

in which:

R^1 is as defined in formula I above; and

$R^4{}_b{}'$ and $R^5{}_a{}'$ are either the same or different and selected from halo, C_{1-6} alkyl, C_{2-6} alkenyl, amine, SO_3H, optionally substituted aryl or optionally substituted heterocyclyl.

(ix) Formula IIIb



IIIb

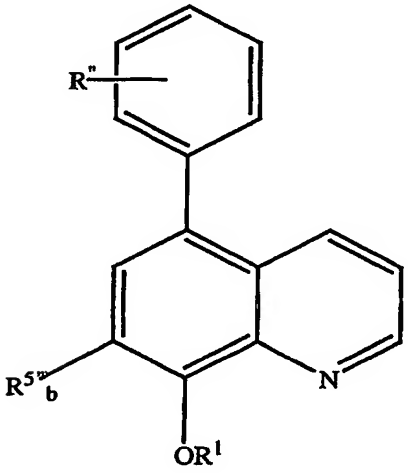
in which:

R^1 is as defined in formula I above;

$R^{4"}_b$ is H or halo; and

$R^{5"}_b$ is optionally substituted aryl or optionally substituted heterocyclyl.

(x) Formula IVb



IVb

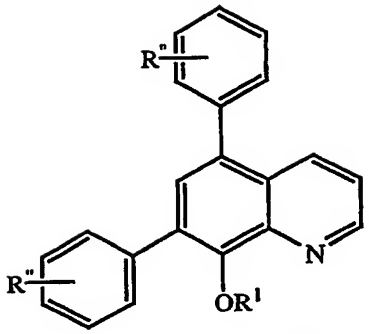
in which:

R^1 is as defined in formula I above;

$R^{"}$ is C_{1-6} alkoxy, halo, C_{1-6} alkyl, C_{2-6} alkenyl or C_{1-6} haloalkyl; and

$R^{5"}_b$ is H or halo.

(xi) Formula Vb



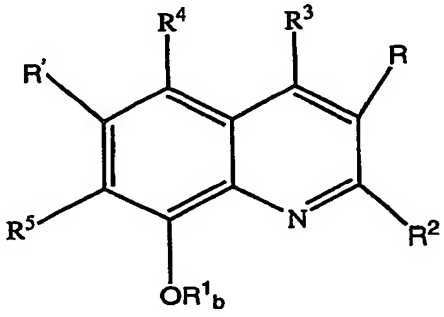
Vb

in which

R^1 is as defined in formula I above; and

R'' is as defined in formula IVb above.

(xii) Formula VIb



VIb

in which:

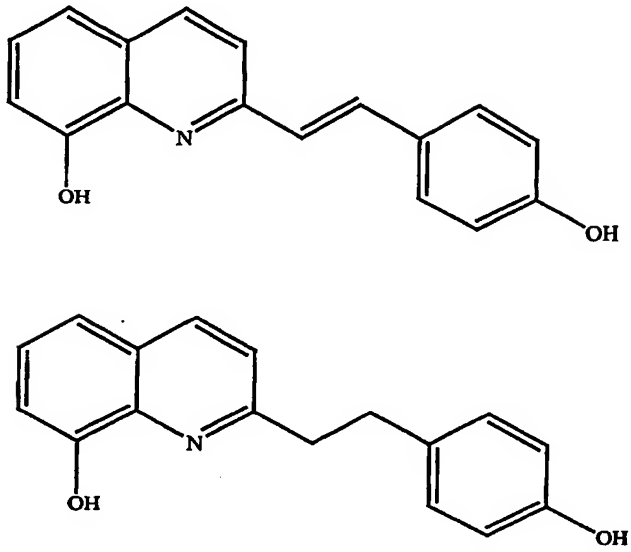
R^2 to R^5, R and R' are as defined in formula I above; and

$R^1{}_b''$ is optionally substituted C_{1-6} alkyl, optionally substituted aryl, optionally substituted aryl acyl, C_{1-6} alkyl acyl or optionally substituted heterocyclyl.

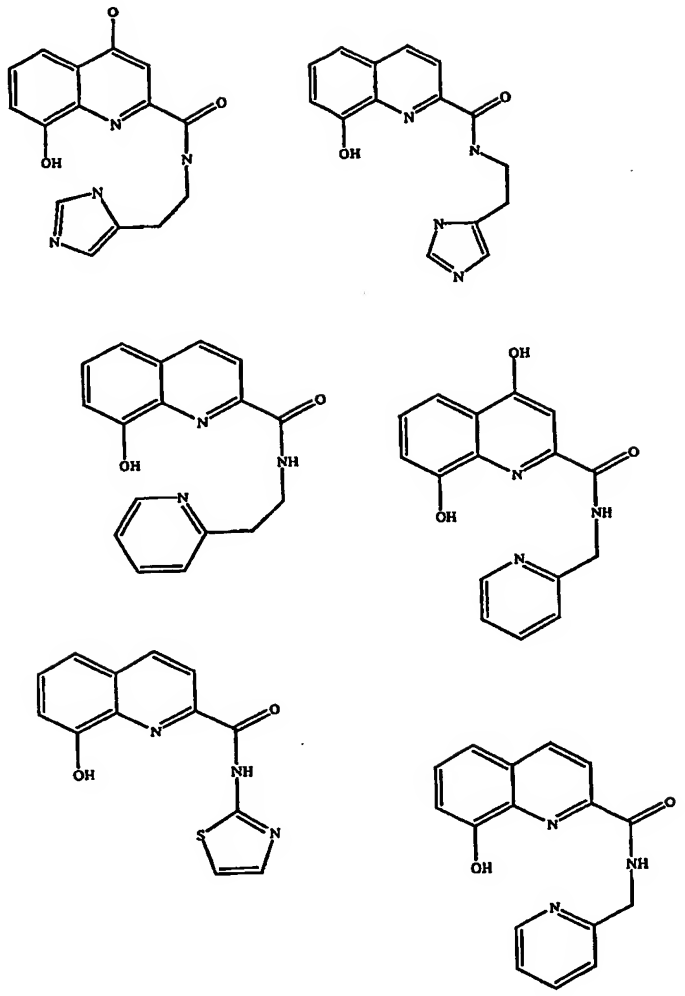
Formula VIb represents compounds in which the 8-hydroxyl group on the quinoline is blocked to form a prodrug, in particular an ester prodrug. The 8-hydroxy represents a principal site of metabolism for the compound of Formula I: conjugation with glucuronic acid or sulphate gives a hydrophilic species ready to be excreted. Such conjugates probably do not pass the blood brain barrier. The ester prodrug may protect the compound of Formula I from conjugation. Esterases integral to the blood brain barrier may then release the C8-hydroxy on passage through that barrier activating the compound for its role in the CNS.

Particularly preferred compounds in view of their neurological activity are shown below:

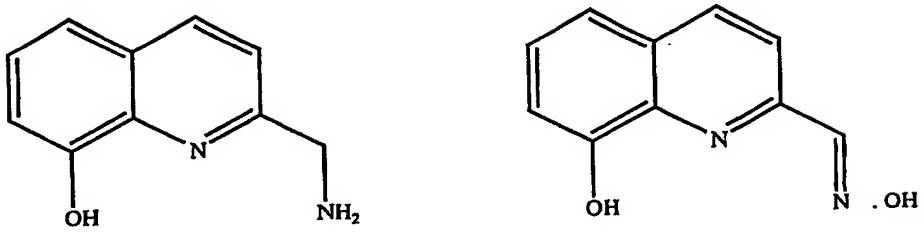
Formula IIa



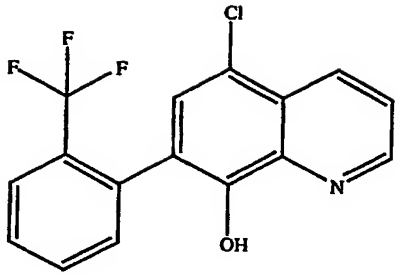
Formula IIIa



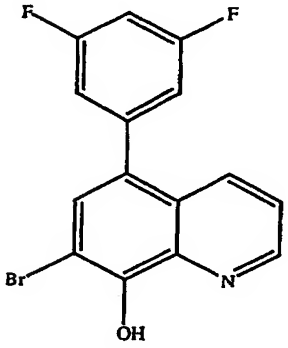
Formula IVa



Formula IIIb



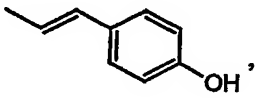
Formula IVb



In a further aspect, the invention provides a pharmaceutical or veterinary composition comprising the compound of formula I as defined above, together with a pharmaceutically or veterinarily acceptable carrier.

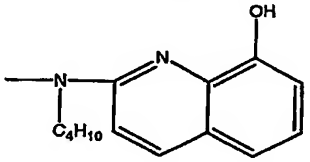
Some of the compounds of formula I are novel *per se*.

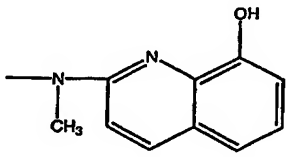
Accordingly, the invention provides a compound of formula II which is a compound of formula I with the provisos that:

(a) when R^1 and R^3 to R^7 are H, then R^2 is not H, methyl, 

,

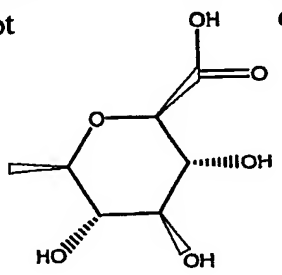
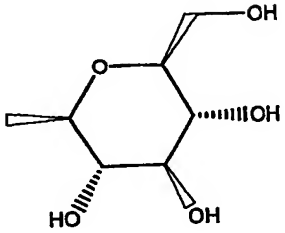
CO_2H, CN, $CONCH_2CO_2H$, $COCH_3$, CH_2NH_2, CNOH, (pyrid-2-yl), 2-hydroxyphenyl, $CHNNH_2$, NH-(pyrid-2-yl),



,


or SO_3H;

(b) when R^1 and R^4 to R^7 are H, then R^3 is not OH and R^2 is not CO_2H; and

(c) when R^1 to R^3, R^6 and R^7 are H, then (i) when R^5 is I, R^4 is not Cl, SO_3H or I; (ii) when R^5 is H, R^4 is not SO_3H, NH_2 or Cl; (iii) R^4 and R^5 are both not Cl, Br or CH_3; and (iv) when R^2 to R^7 are H, then R^1 is not  or 

The compound of formula II defined above may be prepared using the processes described in detail hereinafter.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The term "alkyl" used either alone or in compound words such as "optionally substituted alkyl" "haloalkyl" or "alkyl acyl" refers to straight chain, branched chain or cyclic hydrocarbon groups having from 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms, more preferably 1 to 4 carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

The term "alkenyl" used either alone or in compound words such as "optionally substituted alkenyl", denotes linear, branched or mono- or poly-cyclic radicals having at least one carbon-carbon double bond of 2 to 20 carbon atoms, preferably 2 to 14 carbon atoms, more preferably 2 to 6 carbon atoms. Examples of alkenyl radicals include allyl, ethenyl, propenyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl,1,3-cycloheptadienyl,1,3,5-cycloheptatrienyl,1,3,5,7-cycloocta-tetraenyl and the like.

The term "acyl" used either alone or in compound words such as "optionally substituted acyl", "aryl acyl" or "alkyl acyl", denotes carbamoyl, aliphatic acyl group, acyl group containing an aromatic ring which is referred to as aromatic acyl or an acyl group

containing a heterocyclic ring which is referred to as heterocyclic acyl having 1 to 20 carbon atoms, preferably 1 to 14 carbon atoms. Examples of acyl include carbamoyl; straight chain or branched alkanoyl, such as, formyl, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl or icosanoyl; alkoxycarbonyl, such as, methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, t-pentyloxycarbonyl or heptyloxycarbonyl; cycloalkylcarbonyl, such as, cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentyl, carbonyl or cyclohexylcarbonyl; alkylsulfonyl, such as, methylsulfonyl or ethylsulfonyl; alkoxysulfonyl, such as, methoxysulfonyl or ethoxysulfonyl; aroyl, such as, benzoyl, toluoyl or naphthoyl; aralkanoyl, such as, phenylalkanoyl, for example, phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutyl, phenylpentanoyl or phenylhexanoyl or naphthylalkanoyl, for example, naphthylacetyl, naphthylpropanoyl or naphthylbutanoyl; aralkenoyl, such as, phenylalkenoyl, for example, phenylpropenoyl, phenylbutenoyl, phenylmethacrylyl, phenylpentenoyl or phenylhexenoyl or naphthylalkenoyl, for example, naphthylpropenoyl, naphthylbutenoyl or napththylpentenoyl; aralkoxycarbonyl, such as, phenylakoxycarbonyl, for example, benzyloxycarbonyl; aryloxycarbonyl, such as, phenoxycarbonyl or naphthyloxycarbonyl, aryloxyalkanoyl, such as, phenoxyacetyl or phenoxypropionyl, arylcarbamoyl, such as, phenylcarbamoyl; arylthiocarbamoyl, such as, phenylthiocarbamoyl, arylglyoxyloyl, such as, phenylglyoxyloyl or maphthylglyoxyloyl; arylsulfonyl, such as, phenylsulfonyl or naphthylsulfonyl; heterocycliccarbonyl; heterocyclicalkanoyl, such as, thienylacetyl, thienylpropanoyl, thienylbutanoyl, thienylpentanoyl, thienylhexanoyl, thiazolylacetyl, thiadiazolylacetyl or tetrazolylacetyl, heterocyclicalkenoyl, such as, heterocyclicpropenoyl, heterocyclicbutenoy, heterocyclicpentenoyl or heterocyclichexenoyl; or heterocyclicglyoxyloyl, such as, thiazolylglyoxyloyl or thienylglyoxyloyl.

The term "heterocyclyl group" used either alone or in compound words such as "optionally substituted heterocyclyl" refers to monocyclic or polycyclic heterocyclic groups containing at least one heteroatom atom selected from nitrogen, sulphur and oxygen.

Suitable heterocyclic groups include N-containing heterocyclic groups, such as, unsaturated 3 to 6-membered heteromonocyclic groups containing 1 to 4 nitrogen atoms, for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl or tetrazolyl;

saturated 3 to 6-membered heteromonocyclic groups containing 1 to 4 nitrogen atoms, such as, pyrrolidinyl, imidazolidinyl, piperidino or piperazinyl;

unsaturated condensed heterocyclic groups containing 1 to 5 nitrogen atoms, such as indolyl, isoindolyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl or tetrazolopyridazinyl;

unsaturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, such as, pyranyl or furyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms, such as, thienyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, oxazolyl, isoxazolyl or oxadiazolyl;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, morpholinyl;

unsaturated condensed heterocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, benzoxazolyl or benzoxadiazolyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, thiazolyl or thiadiazolyl;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, thiazolidinyl; and

unsaturated condensed heterocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, benzothiazolyl or benzothiadiazolyl.

Preferably the heterocyclyl is as an unsaturated 5- or 6-membered heteromonocyclic group containing 1 or 3 nitrogen atoms such as imidazolyl, triazolyl, pyrazolyl or pyridinyl; an unsaturated condensed heterocyclic group such as quinolyl or benzothiadiazolyl; an unsaturated 5-membered heteromonocyclyl group containing 1 to 2 sulphur atoms such as thiophenyl; or an unsaturated 5- or 6-membered heteromonocyclyl group containing 1 to 2 sulphur atoms and 1 to 2 nitrogen atoms such as thiazolyl.

The term "aryl" used either alone or in compound words such as "optionally substituted aryl" or "aryl acyl" denotes a carbocyclic aromatic system containing one, two or three rings wherein such rings may be attached together in a pendent manner or may be fused. The term "aryl embraces aromatic radicals such as phenyl, naphthyl, tetrahydronaphthyl, indane and biphenyl. Preferably, the aryl is phenyl.

The term "halo" refers to fluorine, chlorine, bromine or iodine, preferably bromine.

The term "alkylthio" refers to radicals containing a linear or branched alkyl of 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms attached to a divalent sulphur atom. Examples of alkylthio radicals include methylthio, ethylthio, propylthio, butylthio and hexylthio.

The term "alkylsulfinyl" refers to radicals containing a linear or branched alkyl radical, of 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms attached to a divalent -S(=O)- radical. Examples include methylsulfinyl, ethylsulfinyl, butylsulfinyl and hexylsulfinyl.

The term "alkylsulfonyl" refers to radicals containing a linear or branched alkyl radical of 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms attached to a divalent $-SO_2-$

radical. Examples include methylsulfonyl, ethylsulfonyl and propylsulfonyl.

The term "alkoxy" refers to straight chain or branched oxy-containing radicals preferably each having alkyl portions of 1 to about 6 carbon atoms. Examples of alkoxy include methoxy, ethoxy, propoxy, butoxy and *tert*-butoxy.

The term "optionally substituted" refers to a group may or may not be further substituted with one or more groups selected from alkyl, alkenyl, alkynyl, aryl, aldehyde, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, benzylthio, acylthio, phosphorus-containing groups and the like.

The term "antioxidant" is used herein in its broadest sense and refers to a group which has the capacity to react with a reactive oxygen species such as a hydroxyl radical in such a way as to generate a non toxic product. Examples include phenols such as 3,4,5-trimethoxyphenyl and 3,5-di-t-butyl-4-hydroxyphenyl, indole amines such as melatonin and flavonoids. Other examples may be found the literature (Wright, 2001; Karbownik, 2001; Gilgun-Sherki, 2001).

The term "targeting moiety" is used herein in its broadest sense and refers to a group which will facilitate the brain delivery of the drug by way of an active transport mechanism. The targeting moiety is recognised by specific transporter enzymes integral to the bload brain barrier and these transporter enzymes then provide a mechanism for the drug to be imported into the brain. Typically such transporters are sodium dependant and their substrates contain carboxylic acids such as ascorbic acid and L-glutamate. Conjugation of the targeting moiety to the drug is enacted so as to retain the acid moiety. Examples can be found in the literature (Manfredini, 2002, Tamia, 1999).

The term "metal chelator" is used herein in its broadest sense and refers to compounds having two or more donor atoms capable of binding to a metal atom, preferably Cu, Zn or Fe wherein at least two of the donor atoms are capable of simultaneous binding to the metal atom and the resultant metal complex has a thermodynamic stability greater than or equal to that of the Aβ metal ion complex.

The salts of the compound of Formula I or II are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention, since these are useful as intermediates in the preparation of pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include salts of pharmaceutically acceptable cations such as sodium, potassium, lithium, calcium,

magnesium, ammonium and alkylammonium; acid addition salts of pharmaceutically acceptable inorganic acids such as hydrochloric, orthophosphoric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic and hydrobromic acids; or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, trihalomethanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

In addition, some of the compounds of the present invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

By "pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, hydrate or any other compound which, upon administration to the subject, is capable of providing (directly or indirectly) a compound of Formula I or II or an active metabolite or residue thereof.

The term "pro-drug" is used herein in its broadest sense to include those compounds which are converted *in vivo* to compounds of Formula I or II. Use of the pro-drug strategy optimises the delivery of the drug to its site of action, for example, the brain. In one aspect, the term refers to the presence of a C_{1-6} alkyl or arylester moiety which is designed to resist hydroysis until the pro-drug has crossed the BBB, where esterases on the inner surface of the BBB act to hydrolyse the ester and liberate the C8 hydroxyl of the compounds of formula I or II. In a second aspect, the term refers to the attachment at C2 of the 8-hydroxyquinoline core of an antioxidant group, in particular the 3,4,-5trimethoxyphenyl moiety or derivatives thereof. Exposure to the prooxidative environment of the brain will then lead to hydroxylation of the 3,4,5-trimethoxyphenyl group to give a 2-hydroxy-3,4,5-trimethoxyphenyl substituent, the hydroxyl group of which acts to enhance the chelation properties of the compounds of formula I or II.

The term "tautomer" is used herein in its broadest sense to include compounds of Formula I or II which are capable of existing in a state of equilibrium between two isomeric forms. Such compounds may differ in the bond connecting two atoms or groups and the position of these atoms or groups in the compound.

The term "isomer" is used herein in its broadest sense and includes structural, geometric and stereo isomers. As the compound of Formula I or II may have one or more chiral centres, it is capable of existing in enantiomeric forms.

The compositions of the present invention comprise at least one compound of Formula I or II together with one or more pharmaceutically acceptable carriers and optionally other therapeutic agents. Each carrier, diluent, adjuvant and/or excipient must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the

composition and not injurious to the subject. Compositions include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers, diluents, adjuvants and/or excipients or finely divided solid carriers or both, and then if necessary shaping the product.

The term "neurological condition" is used herein in its broadest sense and refers to conditions in which various cell types of the nervous system are degenerated and/or have been damaged as a result of neurodegenerative disorders or injuries or exposures. In particular, compounds of formula I or II can be used for the treatment of resulting conditions, in which damage to cells of the nervous system has occurred due to surgical interventions, infections, exposure to toxic agents, tumours, nutritional deficits or metabolic disorders. In addition, compounds of the formula I or II can be used for the treatment of the sequelae of neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, amylotrophic lateral sclerosis, epilepsy, drug abuse or drug addiction (alcohol, cocaine, heroin, amphetamine or the like), spinal cord disorders and/or injuries, dystrophy or degeneration of the neural retina (retinopathies) and peripheral neuropathies, such as diabetic neuropathy and/or the peripheral neuropathies induced by toxins

The term "neurodegenerative disorder" as used herein refers to an abnormality in which neuronal integrity is threatened. Neuronal integrity can be threatened when neuronal cells display decreased survival or when the neurons can no longer propagate a signal.

Neurological disorders that can be treated with the compounds of the present invention include acute intermittent porphyria; adriamycin-induced cardiomyopathy; AIDS dementia and HIV-1 induced neurotoxicity; Alzheimer's disease; amylotrophic lateral sclerosis; atherosclerosis; cateract; cerebral ischaemia; cerebral palsy; cerebral tumour; chemotherapy-induced organ damage; cisplatin-induced nephrotoxicity; coronary artery bypass surgery; Creutzfeldt-Jacob disease and its new variant associated with "mad cow" disease; diabetic neuropathy; Down's syndrome; drowning; epilepsy and post-traumatic epilepsy; Friedrich's ataxia; frontotemporal dementia; glaucoma; glomerulopathy; haemochromatosis; haemodialysis; haemolysis; haemolytic uraemic syndrome (Weil's disease); haemorrhagic stroke; Hallerboden-Spatz disease; heart attack and reperfusion injury; Huntington's disease; Lewy body disease; intermittent claudication; ischaemic stroke; inflammatory bowel disease; macular degeneration; malaria; methanol-induced toxicity; meningitis (aseptic and tuberculous); motor neuron disease; multiple sclerosis; multiple system atrophy; myocardial ischaemia; neoplasia; Parkinson's

disease; peri-natal asphyxia; Pick's disease; progressive supra-nuclear palsy; radiotherapy-induced organ damage; restenosis after angioplasty; retinopathy; senile dementia; schizophrenia; sepsis; septic shock; spongiform encephalopathies; subharrachnoid haemorrage/cerebral vasospasm; subdural haematoma; surgical trauma, including neurosurgery; thalassemia; transient ischaemic attack (TIA); traumatic brain injury (TBI); traumatic spinal injury; transplantation; vascular dementia; viral meningitis; and viral encephalitis.

Additionally, compounds of the present invention may also be used to potentiate the effects of other treatments, for example to potentiate the neuroprotective effects of brain derived nerve growth factor.

The invention is particularly directed to conditions which induce oxidative damage of the central nervous system, including acute and chronic neurological disorders such as traumatic brain injury, spinal cord injury, cerebral ischaemia, stroke (ischaemic and haemorragic), subharrachnoid haemorrage/cerebral vasospasm, cerebral tumour, Alzheimer's disease, Creutzfeldt-Jacob disease and its new variant associated with "mad cow" disease, Huntington's disease, Parkinson's disease, Friedrich's ataxia, cataract, dementia with Lewy body formation, multiple system atrophy, Hallerboden-Spatz disease, diffuse Lewy body disease, amylotrophic lateral sclerosis, motor neuron disease and multiple sclerosis.

More particularly, the invention is directed to the treatment of neurodegenerative amyloidosis. The neurodegenerative amyloidosis may be any condition in which neurological damage results from the deposition of amyloid. The amyloid may be formed from a variety of protein or polypeptide precursors, including but not limited to Aβ, synuclein, huntingtin, or prion protein.

Thus the condition is preferably selected from the group consisting of sporadic or familial Alzheimer's disease, amyotrophic lateral sclerosis, motor neuron disease, cataract, Parkinson's disease, Creutzfeldt-Jacob disease and its new variant associated with "mad cow" disease, Huntington's disease, dementia with Lewy body formation, multiple system atrophy, Hallerboden-Spatz disease, and diffuse Lewy body disease.

More preferably the neurodegenerative amyloidosis is an Aβ-related condition, such as Alzheimer's disease or dementia associated with Down syndrome or one of several forms of autosomal dominant forms of familial Alzheimer's disease (reviewed in St George-Hyslop, 2000). Most preferably the Aβ-related condition is Alzheimer's disease.

In a particularly preferred embodiment of all aspects of the invention, prior to treatment the subject has moderately or severely impaired cognitive function, as assessed by the Alzheimer's Disease Assessment Scale (ADAS)-cog test, for example an ADAS-cog value of 25 or greater.

In addition to slowing or arresting the cognitive decline of a subject, the methods and compounds of the invention may also be suitable for use in the treatment or prevention of

neurodegenerative conditions, or may be suitable for use in alleviating the symptoms of neurodegenerative conditions. The compounds may be able to provide at least a partial reversal of the cognitive decline experienced by patients. If administered to a subject who has been identified as having an increased risk of a predisposition to neurodegenerative conditions, or to a subject exhibiting pre-clinical manifestations of cognitive decline, such as Mild Cognitive Impairment or minimal progressive cognitive impairment, these methods and compounds may be able to prevent or delay the onset of clinical symptoms, in addition to the effect of slowing or reducing the rate of cognitive decline.

Currently Alzheimer's disease and other dementias are usually not diagnosed until one or more warning symptoms have appeared. These symptoms constitute a syndrome known as Mild Cognitive Impairment (MCI), which was recently defined by the American Academy of Neurology, and refers to the clinical state of individuals who have memory impairment, but who are otherwise functioning well, and who do not meet clinical criteria for dementia (Petersen et al., 2001). Symptoms of MCI include:

(1) Memory loss which affects job skills
(2) Difficulty performing familiar tasks
(3) Problems with language
(4) Disorientation as to time and place (getting lost)
(5) Poor or decreased judgement
(6) Problems with abstract thinking
(7) Misplacing things
(8) Changes in mood or behaviour
(9) Changes in personality
(10) Loss of initiative

MCI can be detected using conventional cognitive screening tests, such as the Mini Mental Status Exam, and the Memory Impairment Screen, and neuropsychological screening batteries.

The term "subject" as used herein refers to any animal having a disease or condition which requires treatment with a pharmaceutically-active agent. The subject may be a mammal, preferably a human, or may be a domestic or companion animal. While it is particularly contemplated that the compounds of the invention are suitable for use in medical treatment of humans, it is also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, ponies, donkeys, mules, llama, alpaca, pigs, cattle and sheep, or zoo animals such as primates, felids, canids, bovids, and ungulates.

Suitable mammals include members of the Orders Primates, Rodentia, Lagomorpha, Cetacea, Carnivora, Perissodactyla and Artiodactyla. Members of the Orders

Perissodactyla and Artiodactyla are particularly preferred because of their similar biology and economic importance.

For example, Artiodactyla comprises approximately 150 living species distributed through nine families: pigs (Suidae), peccaries (Tayassuidae), hippopotamuses (Hippopotamidae), camels (Camelidae), chevrotains (Tragulidae), giraffes and okapi (Giraffidae), deer (Cervidae), pronghorn (Antilocapridae), and cattle, sheep, goats and antelope (Bovidae). Many of these animals are used as feed animals in various countries. More importantly, many of the economically important animals such as goats, sheep, cattle and pigs have very similar biology and share high degrees of genomic homology.

The Order Perissodactyla comprises horses and donkeys, which are both economically important and closely related. Indeed, it is well known that horses and donkeys interbreed.

As used herein, the term "therapeutically effective amount" is meant an amount of a compound of the present invention effective to yield a desired therapeutic response, for example, to prevent or treat a neurological condition.

The specific "therapeutically effective amount" will, obviously, vary with such factors as the particular condition being treated, the physical condition of the subject, the type of subject being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compound or its derivatives.

The compounds of the present invention may additionally be combined with other medicaments to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not eliminate the activity of the compound of formula I or II. It will be appreciated that the compound of the invention and the other medicament may be administered separately, sequentially or simultaneously.

Other medicaments may include, for example, where the condition is a β-amyloid related condition, particularly Alzheimer's disease, an inhibitor of the acetylcholinesterase active site, for example phenserine, galantamine, or tacrine; an antioxidant, such as Vitamin E or Vitamin C; an anti-inflammatory agent such as flurbiprofen or ibuprofen optionally modified to release nitric oxide (for example NCX-2216, produced by NicOx) or an oestrogenic agent such as 17-β-oestradiol.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 20th Edition, Williams & Wilkins, Pennsylvania, USA.

As used herein, a "pharmaceutical carrier" is a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering the compound of formula I or II to the subject. The carrier may be liquid or solid and is selected with the planned manner of

administration in mind. Each carrier must be pharmaceutically "acceptable" in the sense of being compatible with other ingredients of the composition and non injurious to the subject.

The compound of formula I or II may be administered orally, topically, or parenterally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes subcutaneous injections, aerosol for administration to lungs or nasal cavity, intravenous, intramuscular, intrathecal, intracranial, injection or infusion techniques.

The present invention also provides suitable topical, oral, and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compounds of the present invention may be administered orally as tablets, aqueous or oily suspensions, lozenges, troches, powders, granules, emulsions, capsules, syrups or elixirs. The composition for oral use may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to produce pharmaceutically elegant and palatable preparations. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharin. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable preservatives include sodium benzoate, vitamin E, alphatocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate. The tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets.

These excipients may be, for example, (1) inert diluents, such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents, such as corn starch or alginic acid; (3) binding agents, such as starch, gelatin or acacia; and (4) lubricating agents, such as magnesium stearate, stearic acid or talc. These tablets may be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Coating may also be performed using techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

The compound of formula I or II as well as the pharmaceutically-active agent useful in the method of the invention can be administered, for *in vivo* application, parenterally by injection or by gradual perfusion over time independently or together. Administration may be intravenously, intraarterial, intraperitoneally, intramuscularly, subcutaneously, intracavity,

transdermally or infusion by, for example, osmotic pump. For *in vitro* studies the agents may be added or dissolved in an appropriate biologically acceptable buffer and added to a cell or tissue.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, anti-microbials, anti-oxidants, chelating agents, growth factors and inert gases and the like.

Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a disease. "Treating" as used herein covers any treatment of, or prevention of disease in a vertebrate, a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject that may be predisposed to the disease, but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving or ameliorating the effects of the disease, i.e., cause regression of the effects of the disease.

The invention includes various pharmaceutical compositions useful for ameliorating disease. The pharmaceutical compositions according to one embodiment of the invention are prepared by bringing a compound of formula I or II, analogues, derivatives or salts thereof, or combinations of compound of formula I or II and one or more pharmaceutically-active agents into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 20th ed. Williams and Wilkins (2000) and The British National Formulary 43rd ed. (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2002; http://bnf.rhn.net), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical

composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed., 1985).

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units may be tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention may be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of the cytotoxic side effects. Various considerations are described, e.g., in Langer, Science, 249: 1527, (1990). Formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspension. Such excipients may be (1) suspending agent such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; (2) dispersing or wetting agents which may be (a) naturally occurring phosphatide such as lecithin; (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate; (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol; (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a

solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of formula I or II may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The compounds of formula I or II may also be presented for use in the form of veterinary compositions, which may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary compositions include those adapted for:

(a) oral administration, external application, for example drenches (e.g. aqueous or non-aqueous solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feed stuffs; pastes for application to the tongue;

(b) parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; or (when appropriate) by intramammary injection where a suspension or solution is introduced in the udder via the teat;

(c) topical applications, e.g. as a cream, ointment or spray applied to the skin; or

(d) intravaginally, e.g. as a pessary, cream or foam.

Dosage levels of the compound of formula I or II of the present invention are of the order of about 0.5 mg to about 20 mg per kilogram body weight, with a preferred dosage range between about 0.5 mg to about 10 mg per kilogram body weight per day (from about 0.5 gms to about 3 gms per patient per day). The amount of active ingredient that may be combined with the carrier materials to produce a single dosage will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for oral administration to humans may contain about 5 mg to 1g of an active compound with an appropriate and convenient amount of carrier material which may vary from about 5 to 95 percent of the total composition. Dosage unit forms will generally contain between from about 5 mg to 500 mg of active ingredient.

Optionally the compounds of the invention are administered in a divided dose schedule, such that there are at least two administrations in total in the schedule. Administrations are given preferably at least every two hours for up to four hours or longer; for example the compound may be administered every hour or every half hour. In one preferred embodiment, the divided-dose regimen comprises a second administration of the compound of the invention after an interval from the first administration sufficiently long that the level of active compound in the blood has decreased to approximately from 5-30% of the maximum

plasma level reached after the first administration, so as to maintain an effective content of active agent in the blood. Optionally one or more subsequent administrations may be given at a corresponding interval from each preceding administration, preferably when the plasma level has decreased to approximately from 10-50% of the immediately-preceding maximum.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

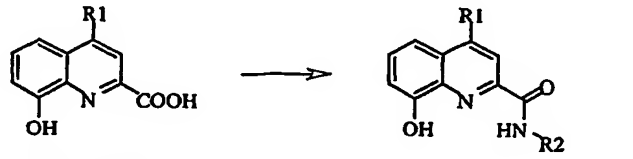
EXAMPLES

The invention will now be described in detail by way of reference only to the following non-limiting examples.

General

8-Hydroxy-quinoline-2-carboxylic acid (Shrader *et al*, 1988) **1**, 8-hydroxy-quinoline-2-carbonitrile (Shrader *et al*, 1988) **2**, 2-chloro-8-hydroxyquinoline (Wang *et al*, 1996; Fleming *et al*, 1971) **3** and 2-Aminomethylthiazole **4** (Dondoni *et al*, 1987, 1996) were prepared according to the literature. The following compounds/reagents were sourced commercially: quinolines: 2-methyl-quinolin-8-ol, 8-hydroxy-quinoline (8-HQ) and 5,7-dibromo-8-hydroxy-quinoline were purchased from Fluka; 4,8-dihydroxy-quinoline-2-carboxylic acid, 5-chloro-7-iodo-8-hydroxy-quinoline and 5,7-diiodo-8-hydroxy-quinoline were purchased from Aldrich; amines: histamine, 2-aminoethylyridine, 2-aminothiazole, 2-(2-aminoethyl)pyridine, 2-(aminomethyl)pyridine, 5-methyl-2-aminothiazole, 2-aminophenol, 1,2-diaminoethane, glycine, 1,2-phenylenediamine, di-(2-picolyl)amine and 2-(2-methylaminoethyl)pyridine were all purchased from Aldrich; aldehydes: 4-imidazolecarboxaldehyde, 2-thiazolecarboxaldehyde and 2-pyridinecarboxaldehyde were all purchased from Aldrich; azoles: pyrazole, imidazole, methylimidazole and 1H-1,2,3-triazole were purchased from Aldrich; boronic acids: 2-(trifluoromethyl)phenylboronic acid, 2-methoxyphenylboronic acid, *o*-tolylboronic acid, 2-fluorophenylboronic acid, 3-methoxyphenylboronic acid, 4-methoxyphenylboronic acid, *m*-tolylboronic acid, 4-(dimethylamino)phenylboronic acid, 2-formylphenylboronic acid, thianaphthene-2-boronic acid, 3,5-difluorophenylboronic acid, 2,4-difluorophenylboronic acid, 3-thiopheneboronic acid, 3-fluorophenylboronic acid, 4-fluorophenylboronic acid and 3-nitrophenylboronic acid were all purchased from Aldrich; and organozinc reagents: 2-pyridylzinc bromide, 2-(methylthio)phenylzinc iodide, 2-(ethoxycarbonyl)phenylzinc iodide and 6-methylpyridylzinc bromide (0.5 M solution in THF) were commercially available (Aldrich). Solvents were analytical grade and used as supplied. THF was distilled from sodium and benzophenone under argon. ^{1}H NMR spectra (δ, relative to TMS) were recorded on a Varian Unity 300 spectrometer unless otherwise indicated; *J*-Values are given in hertz. Mass spectral data were recorded on a Micromass Quattro II mass spectrometer.

Example 1 - Preparation of 8-hydroxy-quinoline-2-carboxylic acid amides (Scheme 1)



Scheme 1

Procedure A:

1,3-Dicyclohexylcarbodiimide (182 mg, 0.87 mmol) was added to a stirred solution of 1-hydroxybenzotriazole hydrate (119 mg, 0.87 mmol) and 8-hydroxy-quinoline-2-carboxylic acid 1 (150 mg, 0.87 mmol) in DMF and dichloromethane (1:1, 10 mL). After 30 min, histamine (182 mg, 0.87 mmol) was added and the mixture stirred at RT for a further 16 h. The volatiles were then removed *in vacuo* and the remaining residue gave, after purification by column chromatography on silica (ethyl acetate/*i*-PrOH/2 N NH_4OH, 6:2:1), 8-hydroxy-quinoline-2-carboxylic acid[2-(1*H*-imidazol-4-yl)-ethyl]-amide A1 as a cream-colored solid.

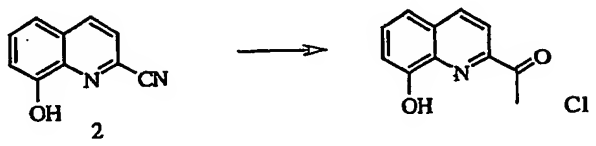
The above reaction was repeated using amines with 1 or 4,8-dihydroxy-quinoline-2-carboxylic acid: histamine gave B1; 2-(2-aminoethyl)pyridine gave A2, 2-(aminomethyl)pyridine gave A5/B2, 2-aminothiazole gave A3, 5-methyl-2-aminothiazole gave A4, 2-aminophenol gave A6, 1,2-diaminoethane gave A7, glycine gave A8/B3, 1,2-phenylenediamine gave B4 and di-(2-picolyl)amine gave A10.

Using A8 as the starting acid, coupling with amines 2-(aminomethyl)pyridine gave B5 and histamine gave B6. Yields and data are given in Table 1.

Procedure B:

8-Hydroxy-quinoline-2-carboxylic acid 1 (100 mg, 0.59 mmol) or 4,8-dihydroxyquinoline-2-carboxylic acid (121 mg, 0.59 mmol) and phosphorus oxychloride (5 mL) were heated under reflux for 1 h, cooled, and concentrated. THF (20 mL) was added to the residue and the mixture cooled (0 °C) before the addition of Et_3N (0.5 mL) and the amine (1.18 mmol). The mixture was allowed to warm to RT. After 16 h, the volatiles were removed *in vacuo* and the resulting residue afforded, after column chromatography on silica, the 8-hydroxy-quinoline-2-carboxylic acid amide. Yields and data are given in Table 1.

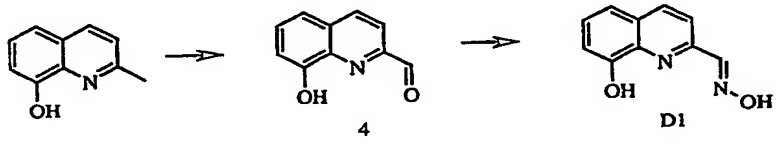
Example 2 – Preparation of 2-Acetyl-8-hydroxy-quinoline C1 (Scheme 2)



Scheme 2

Methylmagnesium bromide (1.2 mL of a 3 M solution in diethyl ether, 3.5 mmol) was added dropwise into a stirred solution of 8-hydroxyquinoline-2-carbonitrile 2 (100 mg, 0.588 mmol) in diethyl ether (10 mL) at –15 °C. The resulting solution was allowed to warm to RT over 2 h and stirred at RT for a further 4 h. The reaction mixture was then quenched with saturated NH_4Cl and extracted with ethyl acetate (10 mL x 3). The extracts were combined, dried (Na_2SO_4) and concentrated to afford the title compound as a pale orange solid (108 mg, 98%) C1. Spectral data of this compound are given in Table 1.

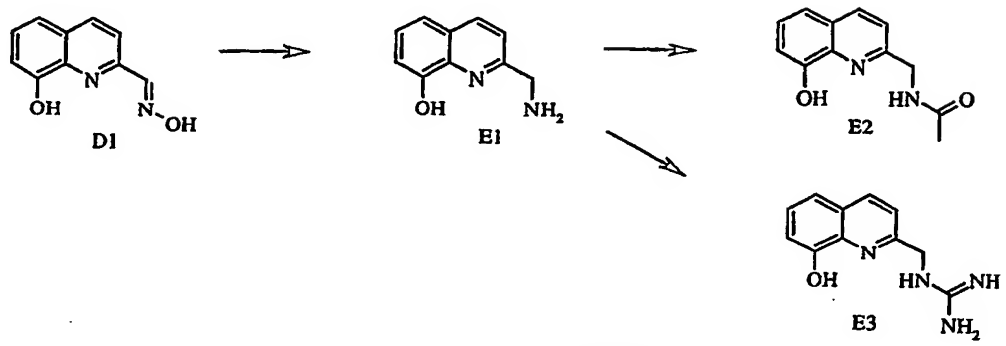
Example 3 – Preparation of 8-Hydroxy-quinoline-2-carboxaldehyde Oxime D1 (Scheme 3)



Scheme 3

A solution of 2-methyl-quinolin-8-ol (536 mg, 3.37 mmol) in dioxane (8 mL) was added dropwise over 3 h into a stirred mixture of SeO_2 (665 mg, 5.99 mmol) in dioxane (25 mL) at 50 – 55 °C. The resulting mixture was then heated at 80 °C for 16 h, cooled, and the solids filtered off. The filtrate was concentrated and the residue purified by column chromatography on silica (dichloromethane/MeOH, 1:0 – 40:1). This afforded 8-hydroxy-quinoline-2-carboxaldehyde 4 as a straw-coloured solid (358 mg, 61%). 4: ^{1}H NMR ($CDCl_3$): δ 10.24 (s, 1 H), 8.34 (d, *J*=8.6, 1 H), 8.22 (br, 1 H), 8.07 (d, *J*=8.6, 1 H), 7.64 (dd, *J*=7.5 and 8.0, 1 H), 7.44 (d, *J*=8.0, 1 H), 7.30 (d, *J*=7.5, 1 H). The mixture of 4 (100 mg, 0.578 mmol), NaOAc (63 mg, mmol), hydroxylamine hydrochloride (60 mg, 0.863 mmol) and water (5 mL) was heated at 100 °C for 15 min. The precipitate was isolated by filtration. This provided the title oxime (ID 969) D1 as an off-white solid (87 mg, 80%); spectral data of this compound are shown in Table 2.

Example 4 - 2-Aminomethyl-quinolin-8-ol E1 (Scheme 4)



Scheme 4

8-Hydroxy-quinoline-2-carboxaldehyde oxime D1 (167 mg, 0.888 mmol) and MeOH (50 mL) was treated under hydrogenolysis conditions (atmospheric H_2, catalytic 10% Pd/carbon) at RT. After 4 h, the catalyst was filtered off and the volatiles removed which afforded 2-aminomethyl-quinolin-8-ol E1 as a light brown solid (126 mg, 82%); spectral data of this compound are given in Table 2.

***N*-(8-Hydroxy-quinolin-2-ylmethyl)-guanidine E3 (Scheme 4)**

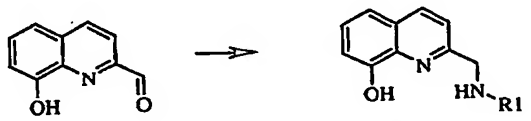
N,N'-Bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (54 mg, 0.174 mmol) was added to a stirred mixture of 2-aminomethyl-quinolin-8-ol E1 (25 mg, 0.144 mmol) in THF (5 mL). After 16 h at RT, the volatiles were removed *in vacuo* and the residue provided, after column chromatography on silica (ethyl acetate/hexane, 1:2), the $(Boc)_2$-derivative of *N*-(8-Hydroxy-quinolin-2-ylmethyl)-guanidine as a colorless solid (52 mg, 87%). A solution of this solid (47 mg, 0.113 mmol) and concentrated hydrochloric acid (0.5 mL) in dioxane (1 mL) was then stirred at RT for 16 h, and concentrated. H_2O (2 mL) was added, the pH adjusted to 8 (conc. NH_4OH) and the mixture concentrated. The solid was dissolved in MeOH and the solution triturated with ethyl acetate. The resulting solid was filtered off and the filtrate was concentrated to a solid. The latter, after column chromatography on silica (ethyl acetate/*i*-PrOH/H_2O, 12:4:1), afforded the title compound E3 as an off-white solid (23 mg, 94%); spectral data are given in Table 2.

2-Acetamidomethyl-quinolin-8-ol E2 (Scheme 4)

A solution of 2-aminomethyl-quinolin-8-ol E1 (30 mg, 0.172 mmol) and Ac_2O (1 mL) in pyridine (2 mL) was stirred at RT overnight and concentrated. Subsequent column chromatography on silica (ethyl acetate) gave 2-acetamido-8-acetoxy-quinoline as a colorless

solid (35 mg, 79%). A solution of 2-acetamido-8-acetoxy-quinoline (33 mg, 0.128 mmol) and K_2CO_3 (50 mg, 0.362 mmol) in MeOH (1 mL) and H_2O (0.5 mL) was stirred at RT for 16 h. Volatiles were removed *in vacuo* and H_2O (2 mL) added. The pH of the mixture was adjusted to 7 (2 N HCl) and the solid was isolated by filtration, washed with H_2O (1 mL x 2) and dried. The title compound **E2** was isolated as a cream solid (21 mg, 76 %); spectral data are given in Table 2.

Example 5 - Reductive amination of 8-hydroxyquinoline-2-carboxaldehyde (Scheme 5)

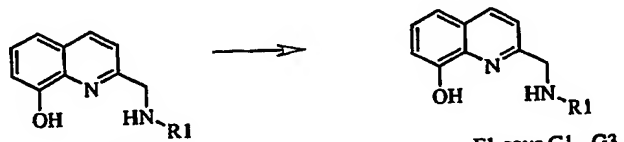


4 F1 - F3

Scheme 5

Sodium triacetoxyborohydride (225 mg, 1.061 mmol) was added to a stirred solution of 8-hydroxy-quinoline-2-carboxaldehyde **4** (200 mg, 1.156 mmol) and histamine (128 mg, 1.152 mmol) in dichloroethane (10 mL). The mixture was left to stir at RT for 16 h, neutralized (aqueous $NaHCO_3$), and concentrated. The resulting residue, after column chromatography on silica (ethyl acetate/*i*-PrOH/2 N NH_4OH, 6:2:1), afforded 2-{[2-(1*H*-imidazol-4-yl)-ethylamino]-methyl}-quinolin-8-ol **F1** as a straw-colored solid (190 mg, 61%). The above method was repeated using other amines: 2-(aminomethyl)pyridine gave **F2** and 2-(2-methylaminoethyl)pyridine gave **F3**, data given in Table 2.

Example 6 - Reductive amination with amines from Example 5 (Scheme 6)



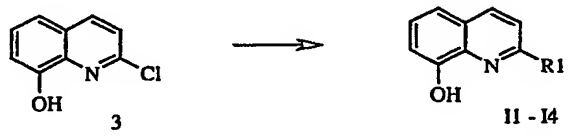
F1 and F2

F1 gave G1 - G3
F2 gave H1 - H3

Scheme 6

Following the procedure of Example 5, aldehydes: 2-imidazolecarboxaldehyde gave **G1/H1**, 2-pyridinecarboxaldehyde gave **G2/H2** and 2-thiazolecarboxaldehyde gave **H3** when treated with **F1** (G series) or **F2** (H series). Results and spectral data are given in Table 2.

Example 7 -2-(Azole)-8-hydroxyquinolines I1 – I4 (Scheme 7)

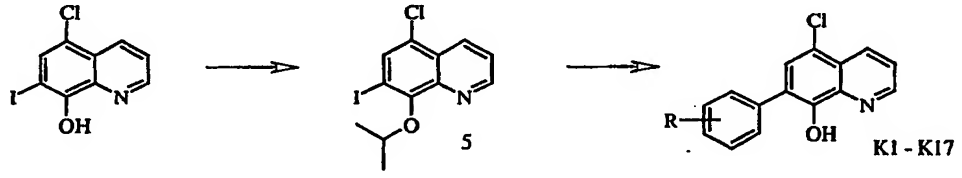


Scheme 7

A mixture of 2-chloro-quinolin-8-ol 3 (80 mg, 0.447 mmol) and pyrazole (152 mg, 2.233 mmol) was heated at 175 °C in a steel autoclave for 48 h. The crude product was then purified by column chromatography on silica (ethyl acetate/hexane, 1:1) to give 2-pyrazol-1-yl-quinolin-8-ol (compound ID 964) I1 as a white solid (68 mg, 72%).

The above procedure was repeated using imidazole, 2-methylimidazole and 1*H*-1,2,3-triazole to give I2, I3 and I4. The crude product for I4 was washed with MeOH (10 mL x 3) to give 2-[1,2,3]triazol-1-yl-quinolin-8-ol (compound ID 994) I4 as an off-white solid (67 mg, 71%). Spectral data of these products are given in Table 3.

Example 8 - Preparation of 5-chloro-7-aryl-8-hydroxyquinolines K1 – K17 (Scheme 8)



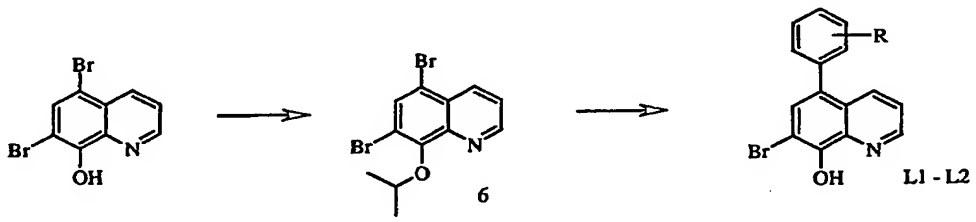
Scheme 8

2-Bromopropane (0.46 mL, 4.90 mmol) was added into a stirred mixture of the 5-chloro-7-iodo-quinolin-8-ol (1.00 g, 3.27 mmol), K_2CO_3 (1.86 g, 13.5 mmol) and DMSO (10 mL). After 16 h at RT, saturated NH_4Cl (10 mL) was added and the mixture extracted with dichloromethane (10 mL x 3). The extracts were combined and concentrated. Diethyl ether (40 mL) was added to the residue and the resulting mixture washed successively with 2 N NaOH, H_2O and brine, and dried (Na_2SO_4). Subsequent column chromatography on silica (ethyl acetate/hexane, 1:1) afforded 5-chloro-7-iodo-8-isopropoxy-quinoline **5** as a solid (1.06 g, 93%). **5**: ^{1}H NMR ($CDCl_3$): δ 8.93 (dd, *J*=1.5 and 4.2, 1 H), 8.52 (dd, *J*=1.5 and 8.4, 1 H), 7.98 (s, 1 H), 7.53 (dd, *J*=4.2 and 8.4, 1 H), 5.38 (m, 1 H), 1.43 (d, *J*=6.0, 6 H). To a stirred mixture of 5-chloro-7-iodo-8-isopropoxy-quinoline **5** (200 mg, 0.58 mmol), phenylboronic acid (77 mg, 0.62 mmol), 2 N Na_2CO_3 (7.2 mL), EtOH (1.2 mL) and benzene (6 mL) was added, under a blanket of argon, $Pd(PPh_3)_4$ (20

mg). The mixture was stirred under reflux for 16 h, cooled and concentrated. This provided, after column chromatography on silica (ethyl acetate/hexane, 1:9), 5-chloro -7-phenyl-8-isopropoxy-quinoline as a yellow solid. To a stirred solution of the 8-isopropoxy-quinoline (0.339 mmol) in dichloromethane (2 mL) at –78 °C was added BCl_3 (1.36 mL of a 1 M solution in dichloromethane, 1.36 mmol). After 2 h, the reaction mixture was allowed to warm to RT and stirred for a further 2 h. MeOH (5 mL) was added and the mixture was concentrated to dryness. This process was repeated four times. Further washing of the remaining residue with diethyl ether (2 mL x 3) provided **K1** in 91% yield. Data in Table 4.

In a similar fashion, reaction of **5** with boronic acids: 2-(trifluoromethyl)phenylboronic acid, 2-methoxyphenylboronic acid (Note cleavage to the 2-hydroxyphenyl derivative), *o*-tolylboronic acid, 2-fluorophenylboronic acid, 3-methoxyphenylboronic acid, 4-methoxyphenylboronic acid, *m*-tolylboronic acid, 4-(dimethylamino)phenylboronic acid, 2-formylphenylboronic acid, thianaphthene-2-boronic acid, 3,5-difluorophenylboronic acid, 2,4-difluorophenylboronic acid, 3-thiopheneboronic acid, 3-fluorophenylboronic acid, 4-fluorophenylboronic acid and 3-nitrophenylboronic acid; and isopropoxy cleavage with BCl_3 gave 5-chloro-7-aryl-8-hydroxyquinolines **K2**-**K17**. Data in Table 4.

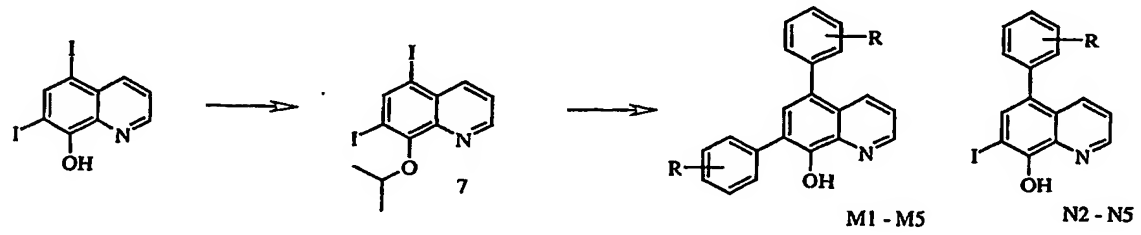
Example 9 - Preparation of 5-aryl-7-bromo-8-hydroxyquinolines L1 – L2 (Scheme 9).



Scheme 9

Reaction of 5,7-dibromo-quinolin-8-ol with 2-bromopropane following the method described in Example 8 gave 5,7-dibromo -8-isopropoxy-quinoline **6** (97%): ^{1}H NMR ($CDCl_3$): δ 8.94 (dd, *J*=1.5 and 4.2, 1 H), 8.48 (dd, *J*=1.5 and 8.4, 1 H), 8.00 (s, 1 H), 7.52 (dd, *J*=4.2 and 8.4, 1 H), 5.22 (m, 1 H), 1.43 (d, *J*=6.1, 6 H); mass spectrum: *m/z* 344, 346, 348 (M^+ + 1, 50, 100 and 50%, respectively). Reaction of **6** with aryl boronic acids, and cleavage of the isopropoxy group following the method outlined in Example 8 gave compounds **L1** and **L2** (data in Table 4).

Example 10 - Preparation of 5,7-diaryl-8-hydroxyquinolines M1 – M5 and 5-aryl-7-iodo-8-hydroxyquinolines N2 – N5 (Scheme 10)



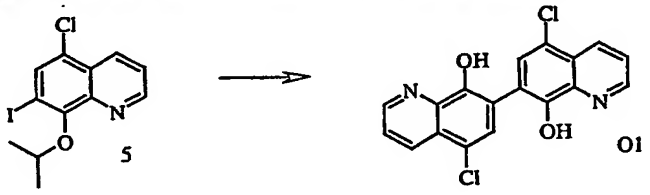
Scheme 10

Preparation of 5,7-diaryl-8-hydroxyquinolines M1 – M5 and 5-aryl-7-iodo-8-hydroxyquinolines N2 – N5 (Scheme 10) .

Reaction of 5,7- diiodo -quinolin-8-ol with 2-bromopropane following the method described in Example 8 gave 5,7-dibromo -8-isopropoxy-quinoline 7 (93%): ^{1}H NMR ($CDCl_3$): δ 8.86 (dd, *J*=1.5 and 4.4, 1 H), 8.46 (s, 1 H), 8.33 (dd, *J*=1.5 and 8.5, 1 H), 7.49 (dd, *J*=4.4 and 8.5, 1 H), 5.40 (m, 1 H), 1.43 (d, *J*=6.1, 6 H). To a stirred mixture of 7 (200 mg, 0.51 mmol), phenylboronic acid (143 mg, 1.17 mmol), 2 N Na_2CO_3 (7.2 mL), EtOH (1.2 mL) and benzene (6 mL) was added, under a blanket of argon, $Pd(PPh_3)_4$ (21 mg). The mixture was stirred under reflux for 16 h, cooled and concentrated. This provided, after column chromatography on silica (ethyl acetate/hexane, 1:9), 5,7-diphenyl-8-isopropoxy-quinoline as a yellow solid (157 mg, 91%). Cleavage of the isopropoxy group following the method outlined in Example 8 gave 5,7-diphenyl-8-hydroxy-quinoline M1 in 91% yield. (See table 4 for data).

Reaction of 7 with aryl boronic acids, and cleavage of the isopropoxy group following the method outlined in Example 8 gave compounds M2 – M5 (data in Table 4). In those cases where the boronic acid contained an *ortho* substituent, the Suzuki reaction yielded a mixture of 5-aryl-7-iodo-8-isopropoxyquinolines and 5,7-diaryl-8-isopropoxyquinolines, which could be separated prior to isopropoxy cleavage to provide both 5-aryl-7-iodo-8- hydroxyquinolines N2 - N5 and 5,7-diaryl-8- hydroxyquinolines M2 - M5.

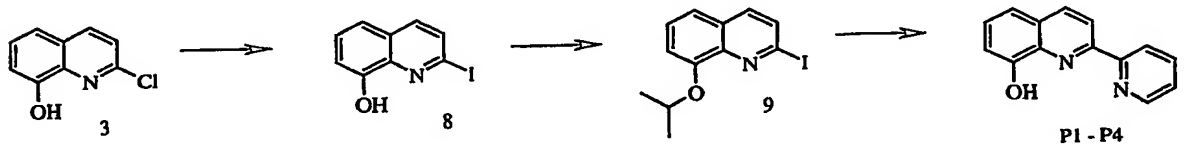
Example 11 - Preparation of 5,5'-Dichloro-8,8'-dihydroxy-7,7'-biquinoline O1 (Scheme 11)



Scheme 11

A solution of **5** (0.576 mmol), bis(pinacolato)diboron (1.1 eqiuv), 2 N Na_2CO_3 (2 mL) and KOAc (3 equiv) was stirred in the presence of a catalytic amount of $PdCl_2$(dppf) in DMF (10 mL) at 80 °C for 3 h. The reaction mixture was then quenched with saturated NH_4Cl and extracted with diethyl ether (10 mL x 3), dried (Na_2SO_4), and concentrated. Column chromatography of the resulting residue (silica; ethyl acetate/hexane, 1:1) afforded 5,5'-dichloro-8,8'-diisopropoxy -7,7'-biquinoline (compound ID 971) as a solid (56 mg, 22%). Cleavage of the isopropoxy groups with BCl_3 following the procedure outlined in Example 8 gave **O1** in 22% yield.

Example 12 - Preparation of 2-aryl-8-hydroxyquinolines P1 – P4 (Scheme 12)



Scheme 12

2-Iodo-quinolin-8-ol 8

Acetyl chloride (0.422 mL, 5.95 mmol) was added dropwise over 20 min into a stirred slurry of 2-chloro-quinolin-8-ol **3** (500 mg, 2.79 mmol), NaI (649 mg, 4.33 mmol) and AcCN (3 mL) at RT.[5] The mixture was then stirred at 35 – 40 °C for 3 h, then overnight at 70 °C, and concentrated. H_2O (10 mL) was added, and mixture was extracted with dichloromethane (10 mL x 3). The extracts were combined and washed successively with a 1:1 solution of saturated $NaHCO_3$ and sodium thiosulfate (5 mL x 2), and H_2O (10 mL x 2), and dried (Na_2SO_4). The residue obtained after solvent removal gave, after column chromatography on silica (ethyl acetate/hexane, 1:8 – 1:3), 2-Iodo-quinolin-8-ol **8** as a white solid (268 mg, 35%) and a 1:2 inseparable mixture of 8-acetoxy-2-iodo-quinoline and 8-acetoxy-2-chloro-quinoline (360 mg)

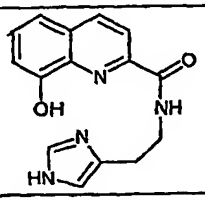
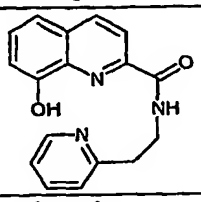
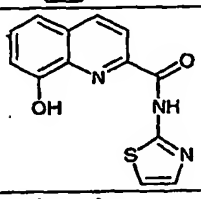
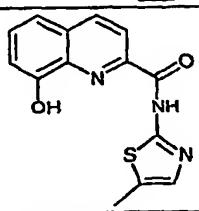
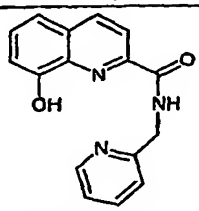
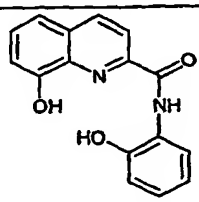
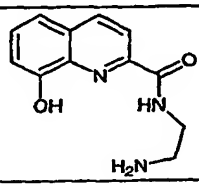
8: ^{1}H NMR ($CDCl_3$): β 7.80 – 7.77 (m, 2 H), 7.49 (dd, *J*=8.1 and 8.1, 1 H), 7.73 (d, *J*=8.1, 1 H), 7.21 (d, *J*=8.1, 1 H), 1.77 (br, 1 H); mass spectrum: *m/z* 272 (M^+ + 1, 100%).

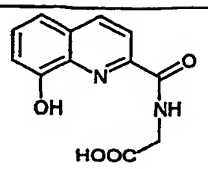
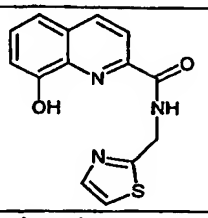
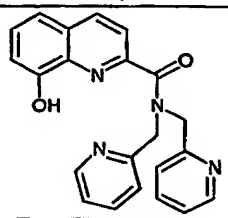
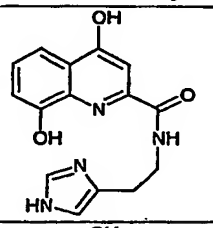
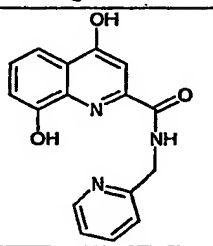
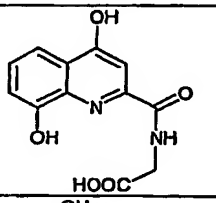
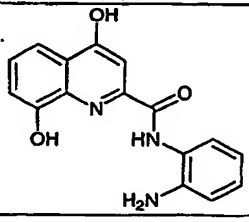
2-(Pyrid-2-yl)-8-hydroxyquinoline M1.

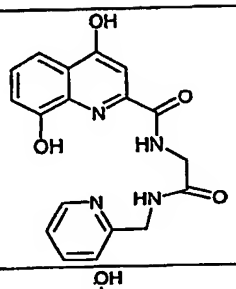
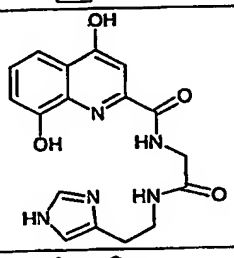
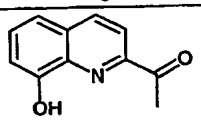
Reaction of 2-iodo-quinolin-8-ol 8 with 2-bromopropane following the method described in Example 8 gave 2-iodo-8-isopropoxyquinoline 9 in 84% yield. 9: ^{1}H NMR ($CDCl_3$): δ 7.75 – 7.67 (m, 2 H), 7.45 (dd, *J*=7.0 and 8.0, 1 H), 7.33 (dd, *J*=1.2 and 8.0, 1 H), 7.12 (dd, *J*=1.2 and 7.0, 1 H), 4.80 (m, 1 H), 1.49 (d, *J*=5.9, 6 H). To a stirred solution of 9 (29 mg, 0.093 mmol) and $PdCl_2(PPh_3)_2$ (5 mg) in THF (2.5 mL) under an argon atmosphere at RT was added dropwise over 5 min 2-pyridylzinc bromide (0.370 mL of a 0.5 M solution in THF, 0.185 mmol). After 2 h, saturated NH_4Cl (5 mL) was added and the mixture extracted with dichloromethane (10 mL x 3). The combined extracts were washed with H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. Subsequent column chromatography on silica (dichloromethane/MeOH, 19:1) gave 2-(pyrid-2-yl)-8-isopropyloxyquinoline as a yellow solid. The isopropyl ether was cleaved according to the procedure of Example 8, to give 2-(Pyrid-2-yl)-8-hydroxyquinoline P1 (22 mg, 89%) (data in Table 5).

This reaction was repeated using: 2-(methylthio)phenylzinc iodide, 2-(ethoxycarbonyl)phenylzinc iodide and 6-methylpyridylzinc bromide to give P2, P3 and P4. Spectral data tabulated (Table 5).

Table 1 Data for Examples 1 and 2.

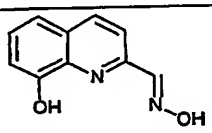
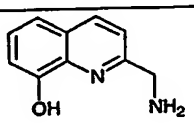
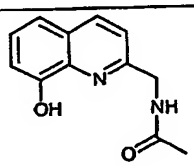
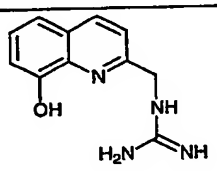
Product ID	Method of Preparation[a]	Product	Yield (%)	^{1}H NMR data	Mass spectral data
A1	A		52	($CDCl_3$/DMSO-d_6, 19:1): δ 9.81 (m, 1 H), 8.30 – 8.23 (m, 2 H), 7.91 (d, *J*=9.0, 1 H), 7.86 (s, 1 H), 7.68 (d, *J*=9.0, 1 H), 7.51 (m, 1 H), 7.36 (m, 1 H), 7.19 (d, *J*=4.0, 1 H), 6.94 (s, 1 H), 3.80 (m, 2 H), 3.03 (m, 2 H)	
A2	A		85	($CDCl_3$): δ 9.61 (m, 1 H), 8.60 (d, *J*=5.4, 1 H), 8.27 – 8.18 (m, 2 H), 8.03 (m, 1 H), 7.93 (d, *J*=8.0, 1 H), 7.77 (d, *J*=8.3, 1 H), 7.63 (d, *J*=8.0, 1 H), 7.51 (d, *J*=8.0, 1 H), 7.35 (d, *J*=8.0, 1 H), 7.24 (m, 1 H), 3.90 (m, 2 H), 3.42 (m, 2 H)	294 (M^+ + 1)
A3	A		65	($CDCl_3$): δ 9.53 (m, 1 H), 8.42 – 8.25 (m, 2 H), 7.65 –7.25 (m, 6 H)	272 (M^+ + 1)
A4	A		81	($CDCl_3$/DMSO-d_6, 19:1): δ 10.35 (br, 1 H), 8.38 – 8.29 (m, 2 H), 7.58 (m, 1 H), 7.48 (s, 1 H), 7.40 (d, *J*=8.3, 1 H), 7.27 – 7.20 (m, 2 H), 2.48 (s, 3 H)	286 (M^+ + 1)
A5	A		81	($CDCl_3$): δ 10.54 (t, *J*=4.0, 1 H), 8.72 (br, 1 H), 8.63 (d, *J*=5.6, 1 H), 8.30 – 8.18 (m, 2 H), 7.9 (d, *J*=7.8, 1 H), 7.64 – 7.30 (m, 5 H), 5.10 (m, 2 H)	280 (M^+ + 1)
A6	A		65	($CDCl_3$/DMSO-d_6, 19:1): δ 11.34 (br, 1 H), 8.36 (s, 1 H), 7.59 (m, 1 H), 7.56 (d, *J*=9.0, 1 H), 7.41 (d, *J*=9.0, 1 H), 7.23 (d, *J*=4.0, 1 H), 7.19 (d, *J*=4.0, 1 H), 7.09 (m, 1 H), 6.96 (m, 1 H), 5.00 (br, 2 H)	
A7	A		71	($CDCl_3$/DMSO-d_6, 19:1): δ 10.16 (m, 1 H), 8.60 (br, 1 H), 8.25 (m, 1 H), 7.92 (d, *J*=7.8, 1 H), 7.67 (d, *J*=7.8, 1 H), 7.57 – 7.35 (m, 2 H), 5.20 (br, 2 H), 3.89 (m, 2 H), 2.60 (m, 2 H)	

A8	A		62	($CDCl_3$/DMSO-d_6, 19:1): δ 10.35 (m, 1 H), 8.37 (s, 1 H), 7.81 (m, 1 H), 7.59 – 7.39 (m, 2 H), 4.23 (m, 2 H), 3.60 (br, 2 H)	
A9	B		60	($CDCl_3$): δ 9.01 (m, 1 H), 8.31 (m, 1 H), 8.25 (br, 1 H), 7.77 (d, *J*=3.4, 1 H), 7.55 (dd, *J*=8.0 and 8.0, 1 H), 7.40 (d, *J*=8.0, 1 H), 7.33 (d, *J*=3.4, 1 H), 7.27 (m, 1 H), 7.24 (d, *J*=7.3, 1 H), 5.05 (m, 2 H)	286 (M^+ + 1)
A10	B		63	($CDCl_3$): δ 9.60 (br, 1 H), 8.75 (d, *J*=4.2, 1 H), 8.58 (d, *J*=4.5, 1 H), 8.28 (d, *J*=8.6, 1 H), 8.08 (d, *J*=8.6, 1 H), 7.77 (m, 1 H), 7.68 (m, 1 H), 7.58 – 7.15 (m, 7 H)	371 (M^+ + 1)
B1	A		77	($CDCl_3$/DMSO-d_6, 19:1): δ 9.60 (m, 1 H), 8.29 (s, 1 H), 7.91 (d, *J*=8.3, 1 H), 7.68 – 7.65 (d, *J*=9.0, 1 H), 7.50 - 7.29 (m, 2 H), 7.15 – 7.07 (m, 2 H), 3.40 (m, 2 H), 3.30 (br, 2 H), 3.10 (m, 2 H).	
B2	A		31	($CDCl_3$/DMSO-d_6, 19:1): δ 10.11 (m, 1 H), 9.55 (br, 1 H), 8.63 (d, *J*=4.4, 1 H), 7.95 (m, 1 H), 7.70 – 7.65 (m, 2 H), 7.58 (s, 1 H), 7.45 (m, 1 H), 7.38 –7.34 (m, 2 H), 7.14 (m, 1 H), 4.96 (m, 2 H)	
B3	A		78	($CDCl_3$/DMSO-d_6, 19:1): δ 10.35 (m, 1 H), 8.37 (s, 1 H), 7.81 (m, 1 H), 7.59 – 7.39 (m, 2 H), 4.23 (m, 2 H), 3.60 (br, 2 H)	
B4	A		97	($CDCl_3$/DMSO-d_6, 19:1):δ 10.99 (br, 1 H), 9.63 (br, 1 H), 7.77 (s, 1 H), 7.69 (d, *J*=8.5, 1 H), 7.50 – 7.32 (m, 3 H), 7.18 – 7.05 (m, 2 H), 6.85 – 6.78 (m, 2 H), 4.20 (br, 2 H)	296 (M^+ + 1)

B5	A		51	($CDCl_3$/DMSO-d_6, 9:1): δ 10.02 (m, 1 H), 8.49 (s, 1 H), 7.95 (m, 1 H), 7.72 – 7.60 (m, 3 H), 7.22 – 7.10 (m, 4 H), 4.57 (m, 2 H), 4.22 (m, 2 H), 3.20 (br, 2 H)	352 (M^+ + 1)
B6	A		47	($CDCl_3$/DMSO-d_6, 19:1): δ 9.92 (m, 1 H), 7.67 (d, *J*=8.8, 1 H), 7.53 (m, 1 H), 7.50 – 7.30 (m, 4 H), 7.12 (d, *J*=8.0, 1 H), 6.76 (s, 1 H), 4.09 (m, 2 H), 3.48 (m, 2 H), 2.60 (m, 2 H)	390 (M^+ + 1)
C1			98	($CDCl_3$): δ 8.31 (d, *J*=9.0, 1 H), 8.18 (d, *J*=9.0, 1 H), 8.15 (br, 1 H), 7.60 (dd, *J*=9.0 and 9.0, 1 H), 7.42 (d, *J*=9.0, 1 H), 7.28 (d, *J*=9.0, 1 H), 2.88 (s, 3 H)	

[a]See Experimental Section: A = General Procedure A; B = General Procedure B.

Table 2 Data for Examples 3, 4, 5 and 6

Product ID	Product	Yield (%)	^{1}H NMR data	Mass spectral data
D1		80	($CDCl_3$/DMSO-d_6, 19:1): δ 11.00 (br, 1 H), 8.41 (s, 1 H), 8.16 (d, *J*=8.6, 1 H), 8.03 (d, *J*=8.6, 1 H), 7.47 (m, 1 H), 7.34 (d, *J*=8.3, 1 H), 7.23 (d, *J*=7.5, 1 H), 2.40 (br, 1 H)	
E1		82	($CDCl_3$/DMSO-d_6, 19:1): δ 8.12 (d, *J*=8.0, 1 H), 7.46 – 7.30 (m, 4 H), 7.17 (d, *J*=7.3, 1 H), 4.20 (br s, 2 H), 3.20 (br, 2 H)	
E2		60	($CDCl_3$): δ 8.25 (d, *J*=8.3, 1 H), 7.53 – 7.48 (m, 2 H), 7.38 (d, *J*=8.3, 1 H), 7.30 (d, *J*=7.6, 1 H), 6.70 (br, 1 H), 4.82 (m, 2 H), 3.53 (s, 1 H), 2.14 (s, 3 H)	
E3		82	($CDCl_3$/DMSO-d_6, 19:1): δ 8.89 (br, 1 H), 8.20 (d, *J*=8.5, 1 H), 7.56 (m, 1 H), 7.50 – 7.40 (m, 2 H), 7.33 (d, *J*=8.0, 1 H), 7.21 (d, *J*=7.5, 1 H), 5.30 (br, 1 H), 4.83 (m, 2 H), 2.88 (br s, 3 H)	

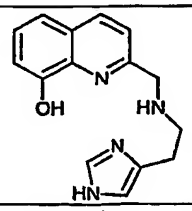
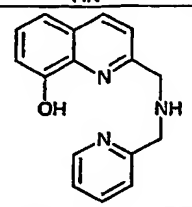
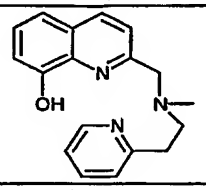
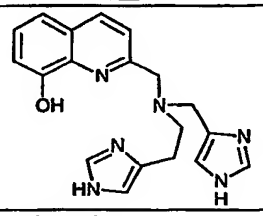
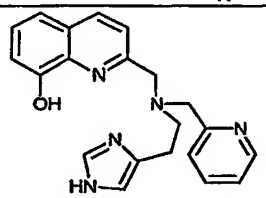
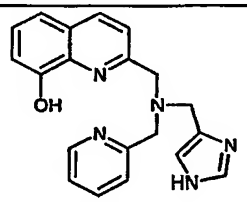
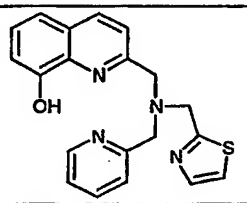
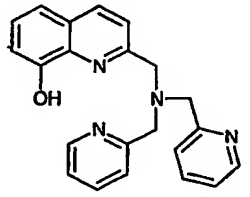
F1		61	($CDCl_3$/DMSO-d_6, 19:1): δ 8.02 (d, *J*=8.6, 1 H), 7.50 (s, 1 H), 7.35 – 7.20 (m, 4 H), 7.06 (d, *J*=7.3, 1 H), 6.74 (s, 1 H), 6.30 (br, 2 H), 4.10 (s, 2 H), 3.03 (m, 2 H), 2.84 (m, 2 H)	
F2		86	($CDCl_3$): □ 8.63 (d, *J*=4.7, 1 H), 8.11 (d, *J*=8.3, 1 H), 7.66 (m, 1 H), 7.46 (d, *J*=8.3, 1 H), 7.41 (d, *J*=7.8, 1 H), 7.34 (d, *J*=8.3, 1 H), 7.28 (d, *J*=8.3, 1 H), 7.22 –7.16 (m, 2 H), 4.18 (m, 2 H), 4.02 (m, 2 H), 2.60 (br, 2 H)	
F3		68	($CDCl_3$): □ 8.53 (d, *J*=4.9, 1 H), 8.06 (d, *J*=8.3, 1 H), 7.59 (m, 1 H), 7.45 (d, *J*=8.3, 1 H), 7.40 (d, *J*=7.8, 1 H), 7.29 (d, *J*=8.3, 1 H), 7.16 (d, *J*=8.3, 1 H), 7.14 (m, 1 H), 3.91 (s, 2 H), 3.08 (m, 2 H), 2.91 (m, 2 H), 2.39 (s, 3 H)	
G1		77	($CDCl_3$/DMSO-d_6, 19:1): δ 8.02 (d, *J*=8.6, 1 H), 7.50 (s, 1 H), 7.35 – 7.20 (m, 4 H), 7.06 (d, *J*=7.3, 1 H), 6.74 (s, 1 H), 6.30 (br, 2 H), 4.10 (s, 2 H), 3.03 (m, 2 H), 2.84 (m, 2 H)	
G2		79	($CDCl_3$/DMSO-d_6, 19:1): δ 8.61 (d, *J*=4.9, 1 H), 8.00 (d, *J*=8.3, 1 H), 7.68 (s, 1 H), 7.59 (dd, *J*=7.5 and 7.5, 1 H), 7.42 – 7.13 (m, 7 H), 6.71 (s, 1 H), 4.04 (s, 2 H), 3.93 (s, 4 H), 3.90 (br, 1 H), 2.89 (br s, 4 H)	
H1		66	($CDCl_3$/DMSO-d_6, 19:1): δ 8.59 (d, *J*=4.8, 1 H), 8.10 (d, *J*=8.5, 1 H), 7.71 – 7.64 (m, 2 H), 7.57 (d, *J*=8.5, 1 H), 7.48 – 7.37 (m, 2 H), 7.32 – 7.14 (m, 3 H), 6.96 (s, 1 H), 3.98 (s, 2 H), 3.84 (s, 2 H), 3.80 (br, 1 H), 3.72 (s, 2 H)	346 (M^+ + 1)
H3		51	($CDCl_3$/DMSO-d_6, 19:1): δ 8.11 (d, *J*=8.3, 1 H), 7.80 – 7.65 (m, 2 H), 7.53 (d, *J*=8.0, 1 H), 7.45 – 7.10 (m, 5 H), 6.76 (s, 1 H), 5.30 (br, 1 H), 4.08 (m, 4 H), 2.94 (m, 4 H)	363 (M^+ + 1)
H2		73	($CDCl_3$): δ 8.57 (m, 2 H), 8.10 (d, *J*=8.5, 1 H), 7.70 – 7.63 (m, 2 H), 7.62 – 7.54 (m, 3 H), 7.41 (dd, *J*=8.0 and 8.0, 1 H), 7.31 – 7.14 (m, 4 H), 4.03 (s, 2 H), 3.94 (s, 4 H), 3.40 (br, 1 H)	357 (M^+ + 1)

Table 3 Data for Example 7.

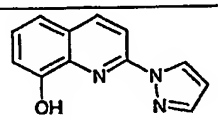
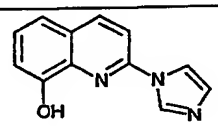
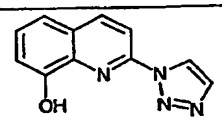
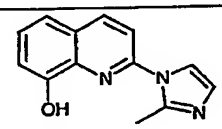
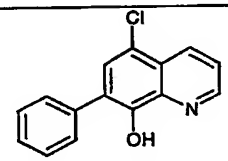
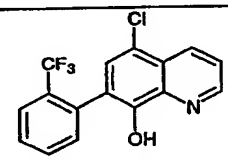
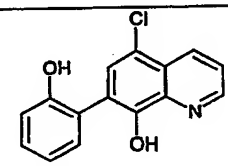
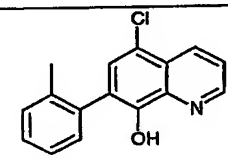
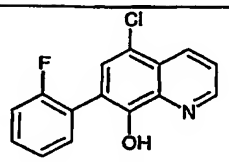
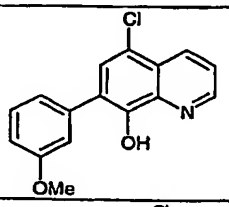
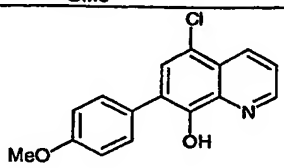
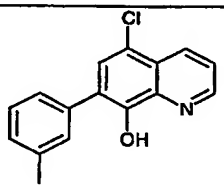
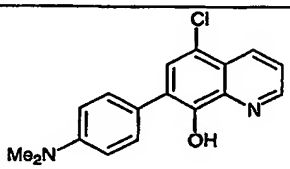
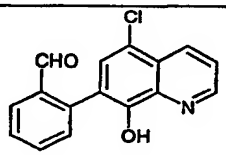
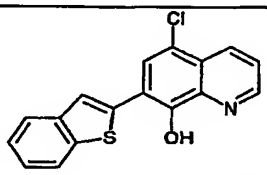
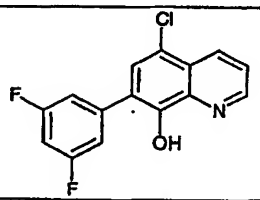
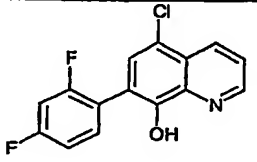
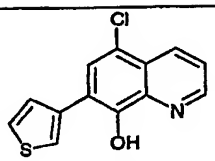
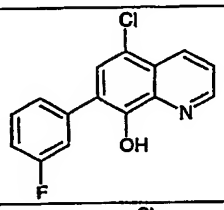
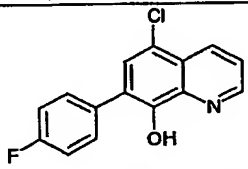
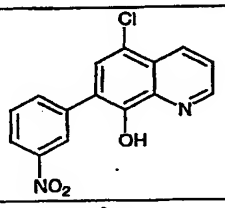
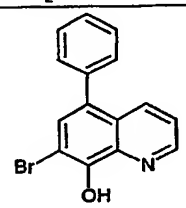
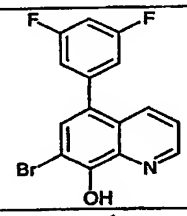
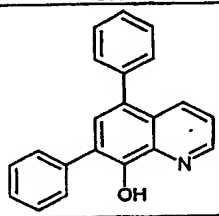
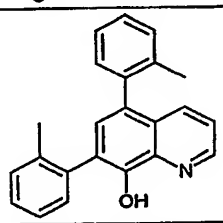
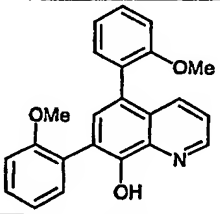
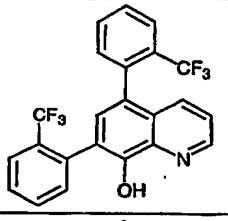
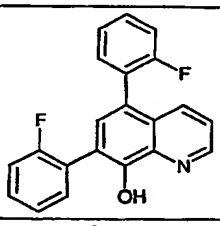
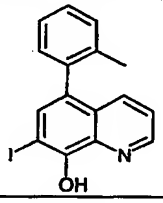
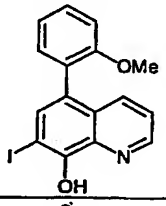
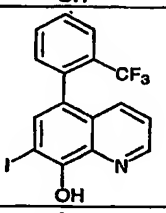
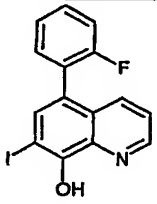
Product ID	Product	Yield (%)	^{1}H NMR data	Mass spectral data
I1		72	($CDCl_3$): δ 8.73 (d, *J*=8.7, 1 H), 8.28 (d, *J*=9.0, 1 H), 8.25 (d, *J*=9.0, 1 H), 7.81 (s, 1 H), 7.66 (s, 1 H), 7.46 – 7.36 (m, 2 H), 7.23 (m, 1 H), 6.56 (m, 1 H)	
I2		75	($CDCl_3$): δ 8.47 (s, 1 H), 8.34 (d, *J*=8.9, 1 H), 7.82 (s, 1 H), 7.70 (br, 1 H), 7.57 (d, *J*=8.9, 1 H), 7.48 (dd, *J*=7.5 and 7.5, 1 H), 7.39 (m, 1 H), 7.28 – 7.25 (m, 2 H)	
I3		71	(DMSO-d_6) (400MHz): δ 8.96 (br, 1 H), 7.58 (d, *J*=9.4, 1 H), 7.54 – 7.49 (m, 2 H), 7.37 (d, *J*=7.8, 1 H), 7.31 (dd, *J*=7.8 and 7.8, 1 H), 7.18 (dd, *J*=1.4 and 7.8, 1 H)	
I4		68	($CDCl_3$):δ 8.34 (dd, *J*=1.5 and 8.8, 1 H), 7.86 (br, 1 H), 7.58 – 7.48 (m, 2 H), 7.42 – 7.40 (m, 2 H), 7.28 (d, *J*=7.8, 1 H), 7.10 (br, 1 H), 2.71 (s, 3 H)	

Table 4 Data for Examples 8, 9, 10 and 11.

Product ID	Product	Yield (%)	^{1}H NMR data	Mass spectral data
K1		89	($CDCl_3$/DMSO-d_6, 19:1):δ 9.16 (m, 1 H), 9.03 (d, *J*=8.6, 1 H), 7.95 (dd, *J*=5.3 and 8.6, 1 H), 7.82 (m, 1 H), 7.58 – 7.42 (m, 3 H), 5.65 (br, 1 H)	256 (M^+ + 1, 100%), 258 (M^+ + 1, 33%)
K2		55	($CDCl_3$/DMSO-d_6, 19:1):δ 9.23 (d, *J*=5.1, 1 H), 9.13 (m, 1 H), 8.02 (m, 1 H), 7.83 (d, *J*=8.0, 1 H), 7.69 (s, 1 H), 7.64 (m, 1 H), 7.41 (d, *J*=7.3, 1 H), 5.60 (br, 1 H)	324 (M^+ + 1, 100%), 326 (M^+ + 1, 33%)
K3		96	(DMSO-d_6)(400MHz): δ 8.99 (d, *J*=4.0, 1 H), 8.57 (d, *J*=8.4, 1 H), 7.78 (dd, *J*=4.0 and 8.4, 1 H), 7.62 (s, 1 H), 7.35 (m, 1 H), 7.18 (m, 1 H), 7.16 – 6.86 (m, 2 H)	270 [(M - H)$^-$, 100%], 272 [(M - H)$^-$, 33%],
K4		90	($CDCl_3$/DMSO-d_6, 19:1): δ 9.19 (d, *J*=5.0, 1 H), 9.13 (d, *J*=8.3, 1 H), 7.96 (dd, *J*=5.0 and 8.3, 1 H), 7.73 (s, 1 H), 7.40 – 7.22 (m, 4 H), 4.10 (br, 1 H), 2.23 (s, 3 H)	270 (M^+ + 1, 100%), 272 (M^+ + 1, 33%)

K5		95	($CDCl_3$/DMSO-d_6, 19:1): δ 9.19 (m, 1 H), 9.12 (d, *J*=8.5, 1 H), 8.00 (dd, *J*=5.1 and 8.5, 1 H), 7.83 (s, 1 H), 7.54 (m, 1 H), 7.47 (m, 1 H), 7.30 (dd, *J*=8.3 and 8.5, 1 H), 7.22 (dd, *J*=8.5 and 8.5, 1 H), 7.00 (br, 1 H)	274 (M^+ + 1, 100%), 276 (M^+ + 1, 33%)
K6		98	($CDCl_3$/DMSO-d_6, 19:1): δ 9.17 (d, *J*=4.7, 1 H), 9.10 (d, *J*=8.3, 1 H), 7.96 (dd, *J*=4.9 and 8.3, 1 H), 7.91 (s, 1 H), 7.43 (dd, *J*=8.1 and 8.1, 1 H), 7.28 – 7.24 (m, 2 H), 6.95 (m, 1 H), 5.00 (br, 1 H), 3.88 (s, 3 H)	286 (M^+ + 1, 100%), 288 (M^+ + 1, 33%)
K7		95	($CDCl_3$/DMSO-d_6, 19:1): δ 9.16 – 9.00 (m, 2 H), 7.92 (dd, *J*=4.9 and 8.6, 1 H), 7.88 (d, *J*=6.3, 1 H), 7.22 – 7.66 (m, 2 H), 7.07 – 7.05 (m, 2 H), 5.70 (br, 1 H), 3.85 (s, 3 H)	286 (M^+ + 1, 100%), 288 (M^+ + 1, 33%)
K8		95	($CDCl_3$/DMSO-d_6, 19:1): δ 9.19 – 9.13 (m, 2 H), 8.05 – 7.93 (m, 2 H), 7.54 – 7.48 (m, 2 H), 7.40 (dd, *J*=7.3 and 7.3, 1 H), 7.27 (d, *J*=7.3, 1 H), 6.65 (br, 1 H), 2.45 (s, 3 H)	
K9		97	($CDCl_3$/DMSO-d_6, 19:1): δ 9.04 (m, 1 H), 8.81 (d, *J*=8.5, 1 H), 7.97 – 7.88 (m, 4 H), 7.82 (m, 1 H), 7.75 (s, 1 H), 4.20 (br, 1 H), 3.26 (s, 6 H)	
K10		68	($CDCl_3$/DMSO-d_6, 19:1): δ 9.24 (s, 1 H), 8.74 (d, *J*=8.3, 1 H), 8.52 (d, *J*=8.0, 1 H), 8.36 (s, 1 H), 8.24 (d, *J*=8.0, 1 H), 7.97 (dd, *J*=7.3 and 7.3, 1 H), 7.82 – 7.70 (m, 3 H), 4.20 (br, 1 H)	
K11		23	(DMSO-d_6)(400MHz): δ 9.47 (d, *J*=6.0, 1 H), 9.09 (d, *J*=8.0, 1 H), 8.51 (s, 1 H), 8.40 (s, 1 H), 8.25 (m, 1 H), 8.10 (m, 1 H), 8.00 (m, 1 H), 7.48 – 7.44 (m, 2 H)	
K12		93	($CDCl_3$/DMSO-d_6, 19:1): δ 9.04 (m, 1 H), 7.82 (m, 1 H), 7.81 (m, 1 H), 7.74 (s, 1 H), 7.38 – 7.30 (m, 2 H), 6.88 (m, 1 H), 4.60 (br, 1 H)	
K13		43	($CDCl_3$/DMSO-d_6, 19:1):δ 9.05 (m, 1 H), 8.81 (d, *J*=8.6, 1 H), 7.81 (m, 1 H), 7.67 (s, 1 H), 7.57 (m, 1 H), 7.07 – 6.95 (m, 2 H), 3.25 (br, 1 H)	

K14		91	($CDCl_3$/DMSO-d_6, 19:1): δ 9.00 (m, 1 H), 8.53 (m, 1 H), 8.16 (m, 1 H), 8.06 (s, 1 H), 7.82 (m, 1 H), 7.75 (dd, *J*=4.2 and 8.5, 1 H), 7.66 (dd, *J*=2.9 and 5.9, 1 H)	
K15		97	($CDCl_3$/DMSO-d_6, 19:1): δ 9.13 (m, 1 H), 9.03 (m, 1 H), 7.93 (m, 1H), 7.87 (m, 1 H), 7.52 – 7.43 (m, 3 H), 7.14 (m, 1H), 5.35 br, 1 H)	
K16		69	($CDCl_3$/DMSO-d_6, 19:1): δ 9.14 (d, *J*=4.9, 1 H), 9.05 (d, *J*=8.6, 1 H), 7.94 (dd, *J*=5.2 and 8.6, 1 H), 7.86 (s, 1 H), 7.75 – 7.69 (m, 2 H), 7.24 – 6.75 (m, 2 H), 5.20 (br, 1 H)	509 (M^+ + 1, 100%), 511 (M^+ + 1, 33%)
K17		41	($CDCl_3$/DMSO-d_6, 19:1): δ 9.06 (m, 1 H), 8.78 (m, 1 H), 8.64 (m, 1 H), 8.26 (m, 1 H), 8.14 (d, *J*=8.0, 1 H), 7.83 (dd, *J*=4.6 and 8.8, 1 H), 7.80 (s, 1 H), 7.72 (dd, *J*=8.0 and 8.0, 1 H), 4.60 (br, 1 H)	
L1		88	($CDCl_3$): δ 9.00 – 8.88 (m, 2 H), 8.02 (s, 1 H), 7.83 (m, 1 H), 7.60 – 7.38 (m, 5 H), 3.80 (br, 1 H)	300 (M^+ + 1, 100%), 302 (M^+ + 1, 100%)
L2		68	($CDCl_3$/DMSO-d_6, 19:1): δ 9.18 (m, 1 H), 8.74 (m, 1 H), 7.92 – 7.84 (m, 2 H), 7.04 – 6.95 (m, 3 H), 5.00 (br, 1 H)	336 (M^+ + 1, 100%), 338 (M^+ + 1, 33%)
M1		91	($CDCl_3$/DMSO-d_6, 19:1): δ 9.15 (m, 1 H), 8.92 (d, *J*=8.3, 1 H), 7.89 (m, 1 H), 7.81 (m, 1 H), 7.78 – 7.72 (m, 2 H), 7.60 – 7.41 (m, 8 H), 4.60 (br, 1 H)	298 (M^+ + 1)
M2		70	($CDCl_3$/DMSO-d_6, 19:1): δ 9.10 (m, 1 H), 9.52 (d, *J*=8.3, 1 H), 7.81 (m, 1 H), 7.59 (s, 1 H), 7.45 – 7.23 (m, 8 H), 3.50 (br, 1 H), 2.25 (s, 3 H), 2.03 (s, 3 H)	

M3		29	($CDCl_3$/DMSO-d_6, 19:1): δδ 9.25 (m, 1 H), 8.59 (d, *J*=8.3, 1 H), 7.88 – 7.81 (m, 2 H), 7.56 – 7.32 (m, 5 H), 7.18 – 7.03 (m, 3 H), 3.90 (br, 1 H), 3.73 (s, 6 H)	
M4		4		432 $(M-H)^-$
M5		39	($CDCl_3$/DMSO-d_6, 19:1): δ 9.13 (m, 1 H), 8.60 (m, 1 H), 7.82 (m, 1 H), 7.76 (s, 1 H), 7.64 – 7.18 (m, 8 H), 3.30 (br, 1 H)	
N2		16	($CDCl_3$/DMSO-d_6, 19:1): δ 9.07 (d, *J*=4.4, 1 H), 8.42 (d, *J*=8.3, 1 H), 8.08 (s, 1 H), 7.81 (dd, *J*=3.2 and 8.3, 1 H), 7.47 – 7.30 (m, 3 H), 7.20 (d, *J*=7.4, 1 H), 5.80 (br, 1 H), 2.01 (s, 3 H)	
N3		46	($CDCl_3$/DMSO-d_6, 19:1): δ 9.06 (d, *J*=3.9, 1 H), 8.53 (d, *J*=8.5, 1 H), 8.08 (s, 1 H), 7.83 (dd, *J*=5.2 and 8.6, 1 H), 7.51 (m, 1 H), 7.27 (m, 1 H), 7.15 (d, *J*=8.3, 1 H), 7.07 (d, *J*=8.3, 1 H), 4.50 (br, 1 H), 3.70 (s, 3 H)	378 $(M^+ + 1)$
N4		79	($CDCl_3$/DMSO-d_6, 19:1): δ 9.08 (m, 1 H), 8.26 (m, 1 H), 8.07 (s, 1 H), 7.89 (m, 1 H), 7.80 (dd, *J*=5.1 and 8.5, 1 H), 7.75 – 7.65 (m, 2 H), 7.36 (m, 1 H), 5.75 (br, 1 H)	416 $(M^+ + 1)$
N5		59	($CDCl_3$/DMSO-d_6, 19:1): δ 9.11 (m, 1 H), 8.54 (m, 1 H), 8.10 (s, 1 H), 7.88 (dd, *J*=5.1 and 8.7, 1 H), 7.54 (m, 1 H), 7.42 – 7.22 (m, 3 H), 5.30 (br, 1 H)	366 $(M^+ + 1)$

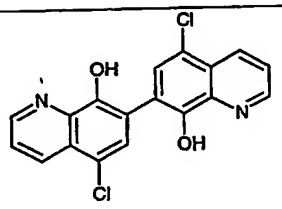
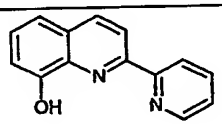
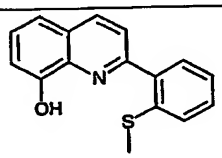
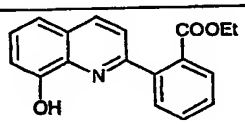
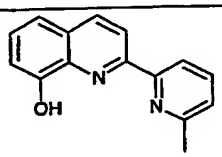
O1		22		355 [(M – H)⁻, 100%], 357 [(M – H)⁻, 66%]

Table 5 Data for the 2-aromatic group-substituted 8-HQ Derivatives (prepared via the Negishi Coupling Reaction)[a]

Product ID	Product	Yield (%)	^{1}H NMR data	Mass spectral data
P1		89	($CDCl_3$): δ 8.98 (d, *J*=3.9, 1 H), 8.60 (d, *J*=8.8, 1 H), 8.40 –8.15 (m, 3 H), 7.75 (m, 1 H), 7.60 (m, 1 H), 7.50 – 7.35 (m, 3 H)	223 (M^+ + 1)
P2		80	($CDCl_3$/DMSO-d_6, 19:1): δ 8.68 (d, *J*=8.3, 1 H), 7.93 (d, *J*=8.3, 1 H), 7.76 – 7.54 (m, 5 H), 7.50 (d, *J*=7.5, 1 H), 7.41 (dd, *J*=7.3 and 7.3, 1 H), 2.50 (br, 1 H), 2.49 (s, 3 H)	268 (M^+ + 1)
P3		33	($CDCl_3$): δ 8.66 (d, *J*=8.8, 1 H), 8.22 (d, *J*=7.1, 1 H), 7.80 – 7.38 (m, 7 H), 4.20 (q, *J*=7.0, 2 H), 1.70 (br, 1 H), 1.18 (t, *J*=7.0, 3 H)	294 (M^+ + 1)
P4		95	($CDCl_3$/DMSO-d_6, 19:1): δ 8.64 (d, J=8.5, 1 H), 8.42 (d, J=7.6, 1 H), 8.28 (d, J=8.5, 1 H), 8.10 (m, 1 H), 7.78 (m, 1 H), 7.48 – 7.16 (m, 3 H), 2.68 (s, 3 H), 2.59 (br, 1 H)	279 (M^+ + 1)

The following assays are used in the assessment of new compounds according to the invention for suitability for use in the methods of the invention.

Fluorometric H_2O_2 assay

A fluorometric assay is used to test for the ability of a test compound to inhibit hydrogen peroxide generation by Aß in the presence of copper based on dichlorofluoroscein diacetate (DCF; Molecular Probes, Eugene OR). The DCF solution (5mM) in 100% dimethyl sulphoxide (previously purged with argon for 2hr at 20°C) is deacetylated in the presence of 0.25M NaOH for 30min and neutralised at pH 7.4 to a final concentration of 1mM. Horseradish peroxidase(HRP) stock solution is prepared to 1µM at pH 7.4. The reactions are carried out in PBS, pH 7.4 in a 96 well plate (total volume =250µl/well). The reactions solutions contain Aß 1-42 at concentrations which may be in the range 50nM to 1µM, copper-glycine chelate (Cu-Gly,

prepared by adding $CuCl_2$ to glycine in the ratio of 1:6 and added to the Aß in the proportion 2Cu-Gly : 1Aß), reducing agents including dopamine (5µM) or ascorbic acid, deacetylated DCF 100µM, and HRP, 0.1µM. 1-10µM EDTA or another chelator may also be present as a control for free copper, but is not required for the assay to function. The reaction mixture is incubated at 37C for 60 min. Catalase (4000 units/ml) and H_2O_2 (1-2.5µM) standards in PBS pH 7.4 may be included as positive controls. Fluorescence is recorded using a plate reader with excitation and emission filters at 485nM and 530nM respectively. H_2O_2 concentration may be established by comparing fluorescence with the H_2O_2 standards. Inhibition of Aß H_2O_2 production is assayed by including a given concentration of test compound(s) in the test wells.

Neurotoxicity Assays

Primary cortical neuronal cultures

Cortical cultures are prepared as previously described (White *et al.*, 1998). Embryonic day 14 BL6Jx129sv mouse cortices are removed, dissected free of meninges and dissociated in 0.025% (wt/vol) trypsin. Dissociated cells are plated in 24 well culture plates (Greiner GmbH, Austria) at a density of 2 x 10^6 cells/mL in MEM with 10% (vol/vol) FCS and 10% (vol/vol) HS. Cultures are maintained at 37°C in 5% CO_2. Prior to experiments, the culture medium is replaced with MEM plus N2 supplements.

Primary cerebellar granule neuronal cultures

Cerebella from post-natal day 5-6 (P5-6) mice are removed and dissected free of meninges and dissociated in 0.025% trypsin. Cerebellar granule neurons (CGN) are plated in 24 well culture plates at 350 000 cells/cm^2 in BME (Gibco BRL) supplemented with 10% FCS, 2 mM glutamine and 25 mM KCl. Gentamycin sulphate (100 µg/mL) is added to all plating media and cultures are maintained at 37°C in 5% CO_2.

Assays for cell viability

MTT assay for cell viability

Cell viability is determined using the MTT assay. Culture medium is replaced with 0.6 mg/mL MTT in control salt solution (Locke's buffer containing 154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM HEPES and 5.6 mM glucose, pH 7.4) for 30 min. The MTT is removed and cells solubilized with dimethyl sulfoxide. 100 µL aliquots are measured with a spectrophotometer at 570 nm.

LDH assay for cell viability

Cell death is determined from culture supernatants free of serum and cell debris using the lactate dehydrogenase (LDH) Cytotoxicity Detection Kit (Boehringer Ingelheim)

according to the manufacturer's instructions.

Assay for neurotoxicity at low Aβ concentration

Cortical cells are prepared following the protocol of White et al. (1998), with the following modifications:

A) On the 5-6th day the medium is changed for Neurobasal medium plus B27 but minus antioxidant.

B) On the 8-9 day the medium is replaced by medium containing test reagents, including Aβ (200-1000 nM), Cu-Gly (400-2000 nM) and dopamine (5-20 μM in PBS).

EDTA (10 μM in PBS) is included throughout to eliminate undesired reactions between free copper and dopamine. However, when testing new drugs, it is advisable not to include EDTA in the Aβ-Cu-Dopamine mixture. For controls, the dopamine volume is replaced with PBS 7.4; the Cu-Gly volume is replaced with water and the Aβ volume is replaced with water.

Aβ peptide solution is prepared by dissolving the peptide in water and centrifuge at 13,000 rpm, for 3-5 min. The supernatant is carefully harvested and its concentration measured by absorbance at 214 nm using the absorbance standard curve.

The following is the mixture sequence and example of approx. volumes of each compound:

For a final volume of 1000 μL, the following sequence is carried out:

Aβ is added using 6.3 μL of Aβ stock (80 μM), to give a final concentration of 500 nM. Thereafter 10 μL of Cu-Gly stock (100 μM) is added to give a final concentration of 1000 nM. 68.7 μL of H_2O and 10 μL of EDTA 1 mM are added, to give a final concentration 10 μM of EDTA. 900 μL of Neurobasal medium plus B27 without antioxidant or Locke's buffer is then added and the solution is mixed. 5 μL of freshly made Dopamine stock (1 mM) is then added to give a final dopamine concentration 5 μM, and the solution is mixed again. The cell medium in each well of the culture is replaced with 250 μL of the mixture, and the cultures are incubated for 16-24 h (37° C). Following incubation, each well is gently washed twice with Locke's buffer, and then the Locke's buffer is replaced with Neurobasal medium (250 μL). Three empty wells are included as background controls.

25 μL of MTS stock is added to each well and incubated for 2-4 hrs at 37° C. The absorbance is then read at 490nM.

Caspase assay

To measure caspase activity in neuronal cultures, growth medium is removed, cells are washed twice with control salt solution (pH 7.4) and ice-cold cell extraction buffer is added directly to the cultures. The extraction buffer consists of 20 mM Tris (pH 7.4), 1 mM sucrose, 0.25 mM EDTA, 1 mM dithiothreitol (DTT), 0.5 mM PMSF, 1% Triton X-100 (Tx-

100) and 1 µg/mL of pepstatin and aprotinin. After incubation for 15 min on ice, the extraction buffer is removed, centrifuged for 5 min at 4°C in a microcentrifuge and 100 µL of supernatant is added to each well of a 96 well plate. 100 µL of 200 µM substrate (either DEVD-pNA, VEID-pNA or IETD-pNA for caspases 3, 6 and 8 respectively) is added to each well to give a final concentration of 100 µM substrate. Plates are incubated at 37°C for 2, 4, 6 or 24 hr and the absorbance is determined at a wavelength of 415 nm (Abs415). The absorbance reading is compared to a known standard of pNA alone.

Annexin V assay

To determine the level of annexin V binding to cells, cultures are washed twice with control salt solution (pH 7.4) followed by the addition of annexin V-FITC at a concentration of approximately 0.5 µg/mL in control salt solution (pH 7.4). Propidium iodide (10 µg/mL) is also added to the cultures at the same time. Cells are incubated in the dark for 30 min at ambient temperature and subsequently washed three times with fresh control salt solution. Analysis of FITC fluorescence (ex. 488 nm, em. 510 nm) is determined using a Leica DMIRB microscope. Photographs are taken with a Leica MPS 60 camera attachment using ASA400 colour film, and negatives are scanned into Adobe Photoshop v2.0.1.

Lipoprotein oxidation assay

Two different assays of metal-mediated lipid peroxidation can be utilized. The first assay involves measuring the oxidative activity of metallated proteins. This is determined by mixing dialyzed metallated or native protein (at designated concentrations) with 0.5 mg/mL LDL for 24 hr (37°C). Lipid peroxidation (LPO) is measured using a lipid peroxidation assay kit (LPO 486, Oxis International Inc. Portland, OR) as per kit instructions. The level of LPO is determined by comparing absorbance (486 nm) with LDL alone (100% LPO). The second assay is used to measure the LPO activity of native proteins in the presence of free, non-protein-bound Cu. This involves adding non-metallated peptides (140 µM) to 0.5 mg/mL LDL together with 20 µM Cu-gly and assaying for LPO as for the metallated proteins. The level of LPO is determined by comparing the absorbance (486 nm) with LDL + Cu-gly (100% LPO). As a negative control, LDL is also exposed to dialysed Cu-gly solutions comparable to those used to Cu-metallate the proteins.

Cytotoxicity induced by Cu-metallated proteins

Proteins or synthetic peptides are mixed with metal-glycine solutions at equimolar or two-fold metal to protein concentration. Metal-protein mixtures are incubated overnight at 37°C and then extensively dialysed (24 hr against two changes of dH_2O (3 L/change) at room temperature) using mini-dialysis cups with a 3,500 kilodalton cut-off (Pierce,

Rockford, IL). Dialysis of proteins against PBS pH 7.4 resulted in metallated proteins with identical activity to dH_2O dialysis.

To determine their neurotoxic effects, metallated proteins, native proteins or peptides are added to two day-old primary cortical neuronal cultures. The cultures are also exposed to Cu-gly (5 or 10 μM) or LDL. Positive control cultures are treated with Cu-gly + LDL or the LPO product, 4-hydroxy-nonenol (HNE, Sigma Chemicals). Cultures are assayed for cell death using the lactate dehydrogenase (LDH) assay kit (Roche Molecular Biochemicals, Nunawading, Australia) according to the manufacturer's instructions.

Acridine orange assay for Aβ-mediated loss of lysosomal acidification

Cultured mouse cortical neurons are treated with Aβ1-42 (20μM) for 16 h and then stained with 5 mg/ml acridine orange (AO) for 5 min at 37°C. 15 min at 37°C. The AO-induced fluorescence is measured with a red filter on a fluorescence microscope. AO is a lysosomotropic weak base which accumulates in the endosomal/lysosomal compartments and displays orange fluorescence during incubation. AO is sequestered inside the lysosomes as long as there is a substantial proton gradient over the lysosomal membranes. Treatment of cells with Aβ1-42 disrupts the lysosomal membrane proton gradient and relocalises AO into the cytosol, as indicated by the loss of orange fluorescence within 16–24 hr.

Brain amyloid solubilisation assay

This assay is performed in order to assess the ability of a test compound to mobilise Aβ from the insoluble to the soluble phase of an extract of tissue from *post mortem* human AD brain.

Up to 0.5 g of plaque-bearing cortex without meninges is homogenized using a DIAX 900 homogenizer (Heudolph and Co, Kelheim, Germany) or other suitable device for three 30-second periods at full speed in 2 ml of ice-cold phosphate-buffered saline, pH 7.4. To obtain the phosphate-buffered saline–extractable fraction, the homogenate is centrifuged at 100,000 x g for 30 min and the supernatant removed. Supernatant, either freeze-dried and resuspended or in unconcentrated form, is dissolved in 200 μl of Tris-Tricine sodium dodecyl sulfate (SDS) sample buffer pH 8.3 containing 8% SDS, 10% 2-mercaptoethanol. Aliquots (10 μl) are then boiled for 10 minutes before SDS–polyacrylamide gel electrophoresis. The insoluble fraction of the cortical samples is obtained by resuspending the initial pelleted sample in 1 ml of phosphate-buffered saline. A 50-μl aliquot of this suspension is then boiled in 200 ml of sample buffer as above.

Tris-Tricine polyacrylamide gel electrophoresis is performed by loading appropriately diluted samples on to 10% to 20% gradient gels (Novex, San Diego, CA) followed by transfer on to 0.2-μm nitrocellulose membrane (Bio-Rad,Hercules, CA). Aβ is detected by

using monoclonal antibody W02, which detects residues 5 through 8, 17 (or another suitable antibody) in conjunction with horseradish peroxidase–conjugated rabbit anti-mouse IgG (Dako, Denmark), and visualized by using enhanced chemiluminescence (eg ECL; Amersham Life Science, Buckinghamshire, UK). Each gel includes three lanes containing 0.5, 1, and 2 ng of synthetic $A\beta_{40}$ (Keck Laboratory, Yale University, New Haven, CT) as reference standards.

Blot films are scanned by using a suitable imaging system such as the UVP gel documentation system, and densitometry performed using suitable software, eg UVP Labworks . The dynamic range of the film/scanner is determined by using a step tablet (No. 911ST600, Kodak, Rochester NY), a calibrated film exposed by the manufacturer to provide steps of known increasing intensity. The quantifiable range of signal intensity for densitometric analysis of the mono- and dimeric Aβ bands is based on the comparison with a curve obtained by scanning and densitometry of the step tablet. Samples in which the signal intensity is low after preliminary assay may be re-assayed by using synthetic standards of lower or higher concentration.

All samples are analyzed at least twice, and gel loadings and dilutions are adjusted to fit within the quantifiable region of the standard curve. The proportion of soluble to insoluble Aβ may be used to determine the efficiency of extraction of the test compound compared with the efficiency of a known compound, such as bathocuproine or clioquinol.

Metal partitioning

To assay effects upon the partitioning of various metals, including zinc and copper, following extraction of brain tissue in the presence of a test compound, soluble and insoluble fractions from an extract of human brain tissue are prepared as for the amyloid solubilisation assay. Metals in the two fractions are analysed by inductively-coupled plasma mass spectrometry, following appropriate pretreatment with nitric acid and/or hydrogen peroxide where necessary.

Effect of administration of agents on Aβ deposits in transgenic animals

Transgenic mouse models are available for a number of neurological disorders, including Alzheimer's disease (Games et al., 1995; Hsiao et al., 1996); Parkinson's disease (Masliah et al., 2000); familial amyotrophic lateral sclerosis (ALS) (Gurney et al., 1994); Huntington's disease (Reddy et al., 1998); and Creutzfeld-Jakob disease (CJD) (Telling et al., 1994). We have found that one of the transgenic models for Alzheimer's disease, the APP2576 tg mouse (Hsiao et al., 1996) also has a high incidence of cataract. These animal models are suitable for testing the methods of the invention.

Transgenic mice of the strain APP2576 (Hsiao et al 1996) are used. Eight to nine month old female mice are selected and divided into groups for treatment.

Mice are sacrificed at intervals, and their brains examined to determine whether the

treatment decreases brain amyloid formation, and to identify the most effective administration protocol. The levels of soluble and insoluble Aβ in the brain and serum are determined using calibrated Western blots. The Aβ plaque burden in the brain is examined immunohistochemically.

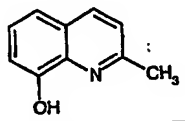
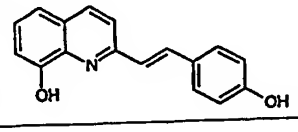
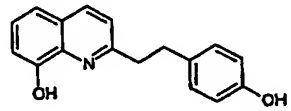
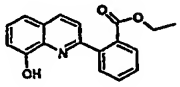
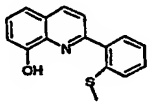
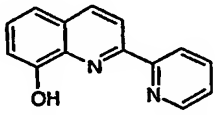
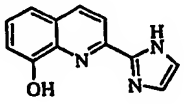
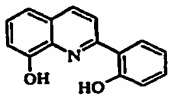
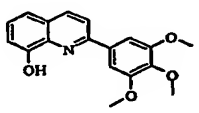
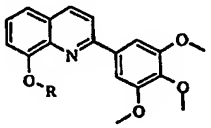
Other mice in each group are tested over a period of up to eight months for cognitive performance, using a Morris water maze according to standard methods. The general health and well-being of the animals is also measured every day by a blinded operator, using a five point integer scale which subjectively rates a combination of features, including motor activity, alertness and general health signs.

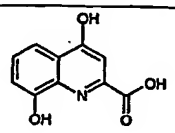
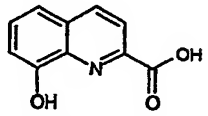
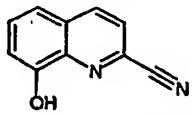
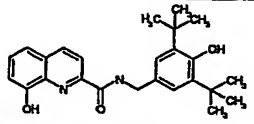
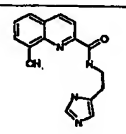
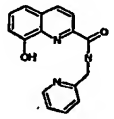
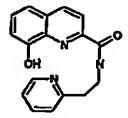
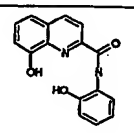
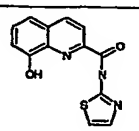
Solubility Assay

Stock solutions of compounds of formula I or II (1mM) were prepared in dimethyl sulfoxide. Compounds which did not dissolve were classed as not soluble (N). The DMSO stock solutions were diluted 1 in 100 into PBS pH 7.4. Compounds which gave a clear solution were classed as soluble (Y), while those compounds which gave a translucent suspension after dissolution in DMSO were classed as "crashed out" (C).

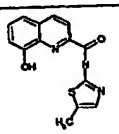
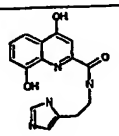
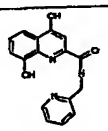
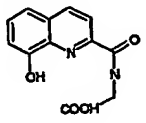
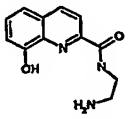
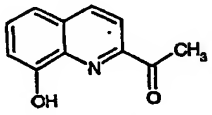
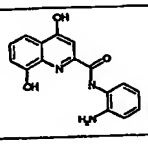
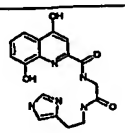
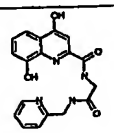
Example 13 - Screening Tests of compound of formula I or II for the treatment of Alzheimer's disease

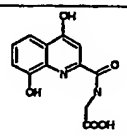
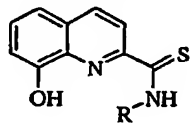
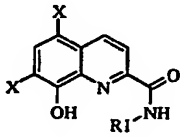
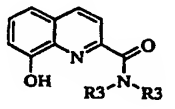
Formula IIa

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
49		826-81-3	Y	2.58	Per_010501		100		
89		189506-06-7	Y	3.7	Per_090401		3		
89					Per_200601		2.5		
89					Tox_150402			74.28	
89					BAS_030602				2
91			Y	3.86	Per_090401		50		
91					Per_200601	>	10		
1004									
1005									
1006									
									
									
									
									

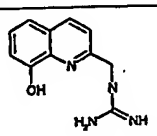
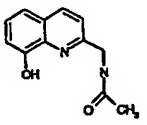
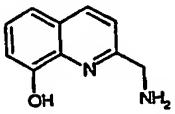
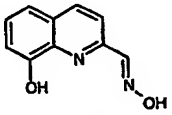
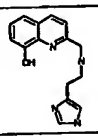
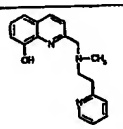
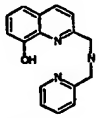
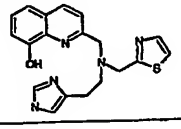
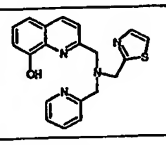
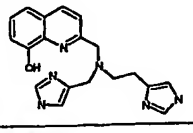
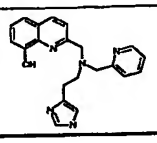
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
52		59-00-7	Y	3	Per_010501	>	100		
57		1571-30-8	Y	2.67	Per_020501		40		
58		6759-78-0	Y	1.95	Per_020501	>	100		
95			Y	6.66	Per_060601		10		
948			Y	1.61	Per_130202		0.19		
948					Per_150202		0.15		
948					Tox_060302			106.66	
948					BAS_030602				3.1
949			Y	2.38	Per_130202		0.43		
949					Per_150202		0.9		
949					Tox_060302			84.82	
949					BAS_030602				3.1
950			Y	2.51	Per_130202		0.25		
950					Per_150202		0.15		
950					Tox_060302			92.8	
950					BAS_030602				2
951			Y	3.26	Per_130202		1.43		
951					Tox_180602			91.86	
952			C	2.47	Per_130202	<	0.81		
952					Per_150202		0.27		
952					Tox_060302			99.52	
952					BAS_030602				2

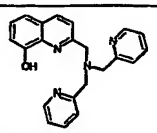
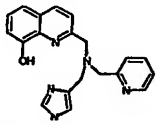
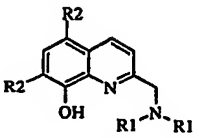
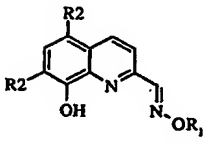
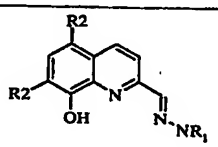
Formula IIIa

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
953			C	2.97	Per_130202	<	4.24		
					Per_150202		0.62		
953					Tox_080402			67.8	
953					BAS_030602				3.1
953									
954			Y	1.93	Per_130202		0.18		
					Per_150202		0.12		
954					Tox_060302			104.9	
954					BAS_030602				2
954									
955			Y	2.71	Per_130202		0.26		
					Per_150202		0.18		
955					Tox_060302			100	
955					BAS_030602				1
955									
956		125686-78-4	Y	1.7	Per_130202	>	10		
					Tox_180602			89	
956									
957			Y	1.42	Per_130202	>	10		
					Tox_180602			95.86	
957									
976		149003-37-2	Y	2.35	Per_280302		3.7		
986			Y	2.8	Per_240502		3.6		
					Tox_180602			81.73	
986									
987			Y	1.08	Per_240502		1.8		
					Tox_180602			89.03	
987									
988			Y	1.76	Per_240502	>	10		
					Tox_180602			93.27	
988									

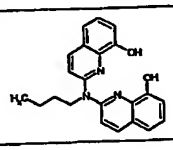
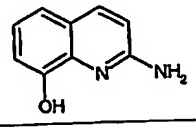
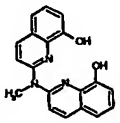
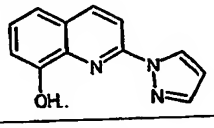
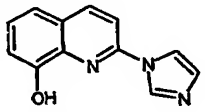
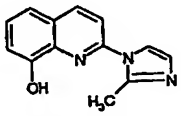
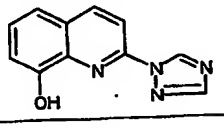
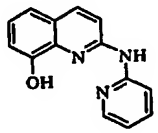
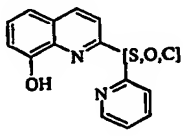
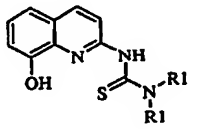
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
992			Y	2.03					
									
									
									

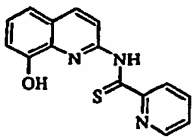
Formula IVa

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
966			Y	0.2	Per_270302		4.3		
966					Tox_180602			88.61	
967			Y	0.89	Per_270302		7.8		
967					Tox_180602			90.69	
968		17018-81-4	Y	1.03	Per_270302		0.26		
968					Tox_180602			97.12	
968					BAS_030602				3.2
969		5603-22-5	Y	2.83	Per_270302		0.54		
969					Tox_180602			94.55	
969					BAS_030602				3.3
989			Y	1.14	Per_310502		0.42		1
989					Tox_180602			43.24	
990			Y	2.51	Per_310502		0.4		1
990					Tox_180602			57.45	
991			Y	1.11	Per_310502		0.47		1
1002				1.95					
1003				2.19					
1008				1.2					
1009				1.88					

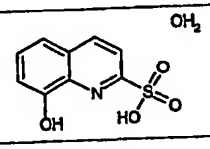
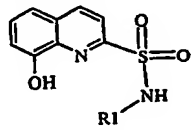
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
1010				2.35					
1011				1.68					
									
									
									

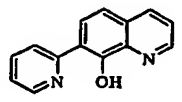
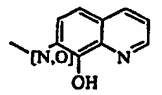
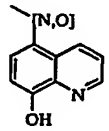
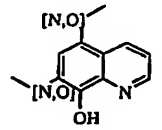
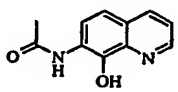
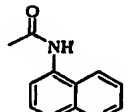
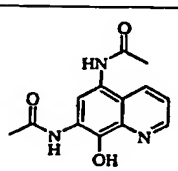
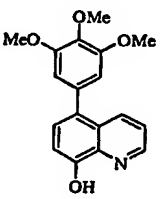
Formula Va

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
53		82361-90-8	Y	6.27	Per_020501		0.3		
					Tox_040202			95.8	
53									
54		70125-16-5	Y	1.75	Per_020501		1		
					Tox_080402			99.57	
54									
56		65165-14-2	Y	4.69	Per_090501		0.7		
					Per_200601		0.25		
56					Tox_080402			24.61	
56					Tox_130502			100.6	
56					BAS_030602				3.1
56									
964			Y	2.97	Per_270302		7.1		
965			Y	1.94	Per_270302	>	10		
993			Y	2.21					
994			Y	1.75					
									
									
									

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
									

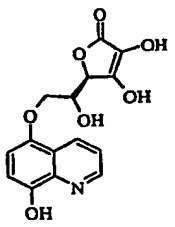
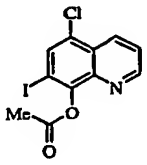
Formula VIa

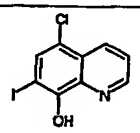
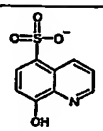
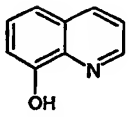
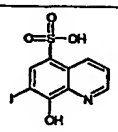
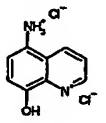
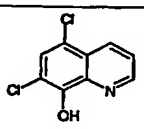
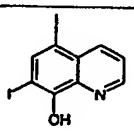
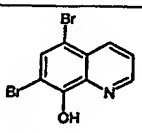
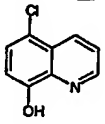
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
50		20946-17-2		0.71	Per_010501		90		
									

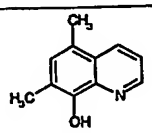
ID	Structure	CAS[1]	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50
							
							
							
							
							
							
							
							

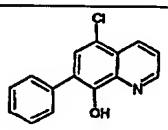
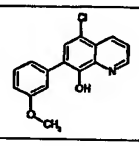
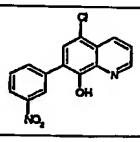
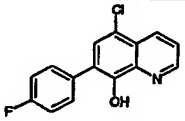
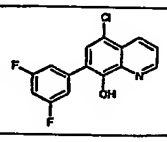
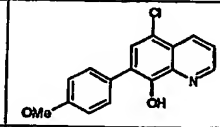
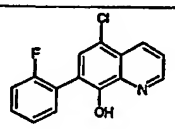
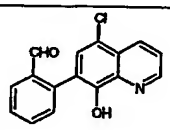
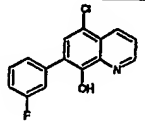
[1]

Formula IIb

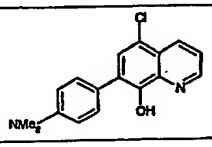
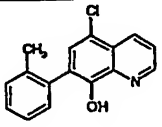
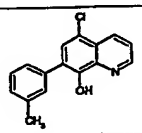
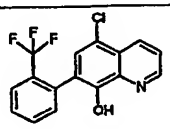
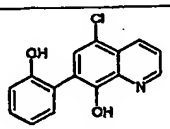
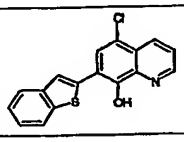
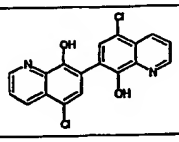
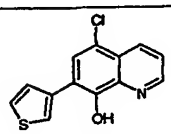
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50
							
							

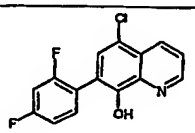
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
1		130-26-7	Y	3.73	Per_061201		0.56		
1					Per_060601		0.5		
41		84-88-8	Y	-0.71	Per_270301		0.5		
41					Tox_110602			81.33	
42		148-24-3	Y	2.08	Per_270401		0.7		
42					Tox_080402			97.66	
42					BAS_030602				3.1
43		547-91-1	Y	0.19	Per_270401		0.6		
43					Tox_110602			91.02	
44		21302-43-2	Y	1.53	Tox_110602			71.05	
44					BAS_030602				3.1
45		773-76-2	Y	3.34	Per_270401		0.7		
45					Per_180601		0.4		
45					Tox_080402			75.19	
45					Tox_110602			66.51	
46		83-73-8	Y	4.14	Per_200301		1		
46					Tox_080402			91.97	
47		521-74-4	Y	3.69	Per_270401		0.9		
47					Per_180601		0.5		
47					Tox_080402			93.59	
47					BAS_030602				3.3
48		130-16-5	Y	2.91	Per_010501		0.8		
48					Per_180601		0.8		
48					Tox_080402			85	

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
59		37873-29-3	Y	3.02	Per_090501		0.7		
59					Tox_150402			84.95	
59					Tox_230402			42.59	

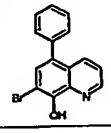
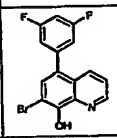
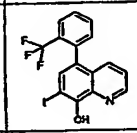
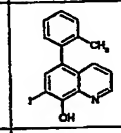
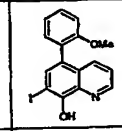
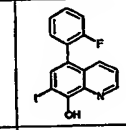
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
808			C	4.3	Per_261001	>	10		
808					Tox_110602			71.89	
810			C	4.23	Per_261001	>	10		
810					Per_131101	<	0.7		
810					Tox_150102			70.7	
810					Tox_290102			90.15	
811			C	4.06	Per_261001	>	10		
811					Tox_110602			78.46	
812			C	4.45	Per_011101	>	10		
812					Tox_110602			75.36	
813			C	4.6	Per_011101	>	10		
813					Per_131101	<	8		
813					Tox_110602			66	
814			C	4.23	Per_011101	<	1.1		
814					Per_131101	>	10		
814					Tox_080402			31.13	
814					BAS_030602				3.1
815			C	4.45	Per_011101	>	10		
815					Tox_110602			53.68	
849			Y	3.67	Per_131101		4.5		
849					Tox_110602			98.83	
850			C	4.45	Per_141101	>	10		
850					Tox_110602			71.28	

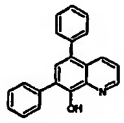
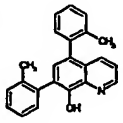
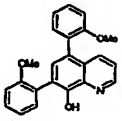
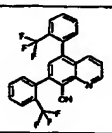
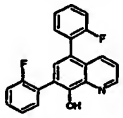
Formula IIIb

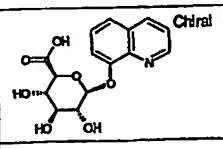
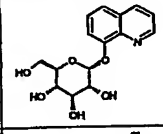
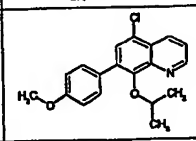
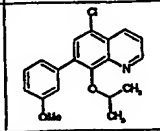
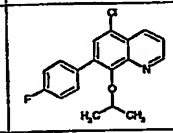
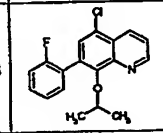
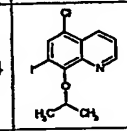
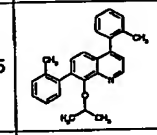
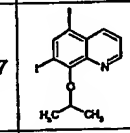
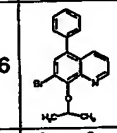
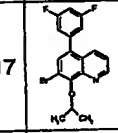
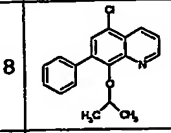
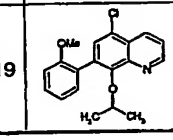
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
851			C	4.47	Per_141101	<	0.7		
851					Tox_150102			84.92	
851					Tox_290102			86.08	
851					BAS_030602				2
854			C	4.5	Per_141101	<	0.78		
854					Tox_150102			100	
854					Tox_290102			71.39	
854					Tox_180602			34.95	
854					BAS_030602				3.2
859			C	4.8	Per_141101	<	0.67		
859					Tox_150102			73.14	
859					Tox_290102			36.01	
859					Tox_180602			34.07	
859					BAS_030602				2
864			Y	5.2	Per_151101		0.77		
864					Tox_290102			93.12	
864					BAS_030602				3.2
947			Y	3.14	Per_130202		1.14		
947					Tox_060302			70.4	
970			C	5.54	Per_270302		6.7		
970					Tox_180602			32.33	
971			C	4.57	Per_280302	>	10		
971					Tox_180602			84.29	
972			C	3.95	Per_280302	>	10		
972					Tox_180602			30.59	

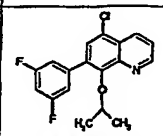
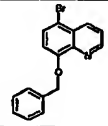
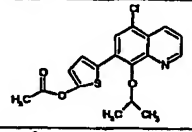
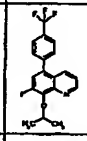
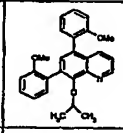
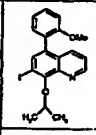
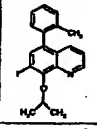
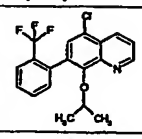
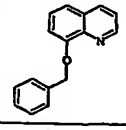
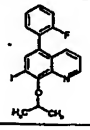
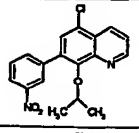
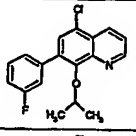
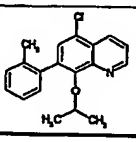
ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
973			C	4.6	Per_280302	>	10		
973					Tox_180602			42.38	

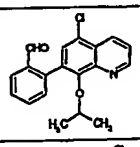
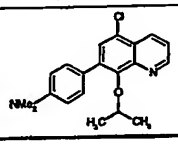
Formula IVb

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
806			C	4.67	Per_261001	<	1.2		
806					Per_131101	<	0.9		
806					Tox_150102			97	
806					Tox_290102			100	
806					BAS_030602				3.2
853			Y	4.97	Per_141101		0.77		
853					Tox_290102			94.79	
853					BAS_030602				2
860			Y	5.76	Per_141101		0.79		
860					Tox_150102			89.58	
860					Tox_290102			64.83	
860					BAS_030602				2
861			C	5.06	Per_141101	<	0.91		
861					Tox_290102			37.83	
861					BAS_030602				3.1
863			C	4.23	Per_151101	<	0.73		
863					Tox_150102			34.97	
863					BAS_030602				2
865			C	5.01	Per_151101	>	10		
865					Tox_180602			34.07	

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
809			C	5.35	Per_261001	<	4		
809					Per_131101	<	1.8		
809					Tox_080402			26.31	
809					BAS_030602				2
852			Y	5.75	Per_141101		2.1		
852					Tox_180602			33.52	
862			C	4.09	Per_151101	<	0.77		
862					Tox_150102			51.52	
862					Tox_290102			52.69	
862					BAS_030602				2
974			Y	7.17	Per_280302		0.6		
975			Y	5.67	Per_280302		3.2		

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
39		14683-61-5	Y		Per_260401		90		
62		29266-96-4	Y		Per_160501		10		
800			C		Per_261001	>	10		
801			C		Per_261001	>	10		
802			C		Per_261001	>	10		
803			C		Per_261001	>	10		
804			C		Per_261001	>	10		
805			C		Per_261001	>	10		
807			C		Per_261001	>	10		
816			C		Per_011101	>	10		
817			C		Per_011101	>	10		
818			C		Per_011101	>	10		
819			C		Per_011101	>	10		

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
820			C		Per_011101	>	10		
821			C		Per_011101	>	10		
822			C		Per_011101	>	10		
823			C		Per_011101	>	10		
824			C		Per_051101	>	10		
825			C		Per_051101	>	10		
826			C		Per_051101	>	10		
827			C		Per_051101	>	10		
855			Y		Per_141101	>	10		
856			C		Per_141101	>	10		
857			C		Per_141101	>	10		
858			Y		Per_141101	>	10		
866			Y		Per_151101	>	10		

ID	Structure	CAS	Sol (Y,C,N)	clogp	Assay ID		Peroxide IC50	Viable 10uM	BAS Score
867			Y		Per_151101	>	10		
868			C		Per_151101	>	10		

Example 14 – Clinical trial of compound of formula I or II for the treatment of Alzheimer's disease

A Phase II clinical trial of the compound of formula I or II for the treatment of AD has been prepared. Inclusion criteria for this Phase II study targeted a region on the ADAS-cog scale (20-45) where patients are moderately demented, still living at home, but anticipating an accelerated deterioration as part of the natural history of AD, over the next 12 months. Because the primary outcome is efficacy, a triple-blind design is chosen.

Several considerations drove our choice of dose. In our previous studies on transgenic mice, doses of 20-30 mg/kg of CQ orally daily for five days per week were markedly effective at inhibiting Aβ accumulation after 2-3 months of treatment. The human equivalent dose of 1500-2250 mg/day is close to the prescribed antibiotic dose of CQ (600 mg po qid). However, this magnitude of dose, administered for months, would raise concerns about SMON toxicity.

As the compound of formula I or II may be conjugated to glucuronide followed by renal excretion, there was some concern that blood levels in the elderly might be elevated by inefficient liver metabolism, constraining the proposed dose even further. Therefore a cautious dose escalation design is chosen, to maximize the chance of detecting a change in outcome measures, while minimizing the risk of adverse effects. The starting dose of 3.3 mg/kg/day, assuming 75 kg average weight, is within the same order of magnitude of the effective dose in the transgenic mouse model, but only about one tenth of the antibiotic dose.

Since there is no data from the transgenic mouse study of the effectiveness of doses less than 20 mg/kg/day, we reasoned that a beneficial effect might require a longer period of treatment than the 9-12 week duration of the mouse study (Cherny et al., 2001). Therefore a trial length of 36 weeks at an average dose which is approximately one-third of what is effective in the transgenic mice is chosen. The final dose of 10 mg/kg/day is half of an effective dose in mice.

Thirty-six patients will be randomised [18 placebo and 18 (the compound of formula I or II)]. Per protocol analyses will be conducted on 33 patients for analyses up to 24 weeks and 32 patients at 36 weeks. Groups will be similar across most demographic, biological and clinical variables at baseline. The effect of treatment was statistically significant in the more-severely affected group (baseline ADAS-Cog $\geq$ 25), but not the less-severely affected group (ADAS-Cog $<$ 25).

METHODS

Ethical issues: In compliance with Australian Commonwealth and Victorian State laws concerning consent from individuals whose cognitive function may be impaired to the extent of being unable to make informed judgements or decisions, "Consent to Special Procedures" administered by the Victorian Civil and Administrative Tribunal will be obtained for each participant. In addition, third party consent will be obtained from all carers, in accordance with Victoria's Guardianship Laws. SMON will be described in detail in the plain language statement, and discussed verbally with both patient and carer at the time of giving consent. As partially effective treatments are now available for AD, it was considered unethical to have the comparison group on placebo only; hence both treatment groups will be placed on donepezil for the duration of the study. The study was approved by the Royal Melbourne Hospital Research Foundation's Clinical Research and Ethics Committee.

Study population:

Criteria for inclusion in the study were:

(a) informed consent;
(b) a diagnosis of probable Alzheimer's disease by NINCDS-ADRDA criteria (McKhann et al., 1984);
(c) Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) score of 20-45 inclusive (Rosen et al., 1984);
(d) Mini Mental State Examination (MMSE) score of 10-24 inclusive (Folstein et al., 1975);
(e) on donepezil hydrochloride 5mg or 10mg for at least 6 months;
(f) relative or carer willing and able to support the trial;
(g) able to complete trial examinations; and
(h) primary sensorial functions intact.

All female patients were postmenopausal.

Patients are to be excluded if they have a potential allergy to the compound of formula I or II; history or clinical evidence of peripheral neuropathy or optic neuropathy; co-existing illnesses or past history which may have affected cognitive function or nerve conduction, including alcohol abuse or dependency; metabolic deficiencies (eg unstable thyroid dysfunction); infections with neurotrophic organisms such as syphilis, HIV, CMV, or EBV; current major depressive episode according to DSM-IV criteria; co-existing illnesses which might confound the adverse event profile, such as diabetes, untreated vitamin B12 or folate deficiency, ulcerative

colitis, Crohn's disease, chronic diarrhoea, or multiple sclerosis; other co-existing medical conditions which might compromise the patient if s/he were to participate in a clinical trial, such as a neoplasm currently active or likely to recur (except non-melanoma skin cancer), history of immunosuppression, gastrointestinal malabsorption, hypertension (BP > 180 mmHg systolic or > 95 mmHg diastolic), cardiac failure (orthopnea, JVP>5cm, or peripheral oedema requiring the prescription of loop diuretics), a history of stroke in the last 6 months or a Hachinski score ≥ 6, haemoglobin >20% below lower limit of normal range, raised white cell count (20% above reference range), neutropenia (white cell count < 2.5), abnormal liver function tests (>50% above reference range), abnormal creatinine clearance (< 75% of reference range), abnormal fasting blood glucose (> 50% above upper limit of normal range), abnormal thyroid function (TSH or T4 >20% outside reference range), or positive hepatitis A,B or C IgM.

The following factors are to be obtained at baseline to determine whether they correlated with outcome measures: age, sex, premorbid IQ (estimated from Natural Adult Reading Text (NART), years of education, serum donepezil hydrochloride, and apolipoprotein E(ApoE) allotype .

Study design: The study is a triple blind, placebo-controlled, randomised design. Thirty-six patients and their carers are to be recruited to participate, with patients being randomised to receive either the compound of formula I or II or placebo; there will be 18 patients in each arm. The duration of the study was 36 weeks. the compound of formula I or II dosage was 125mg twice daily from weeks 0-12, increased to 250mg twice daily from weeks 13-24, and finally, 375mg twice daily from weeks 25-36.

All patients are to be treated with donepezil hydrochloride for at least 6 months prior to recruitment. The dose of donepezil is optimised by each patient's physician to maximize clinical benefit and to minimize side effects. This dose is maintained for the duration of the study, and patients are to be withdrawn from the study if, at regular review, the dose of donepezil required alteration for any reason.

The study medication and placebo are presented as enteric-coated capsules (125mg were blue, 250mg were brown), randomised in blocks of 6. Presentation after increase to 250mg twice daily was as 2 X 125mg per dose; after increase to 375mg, presentation was twice daily 1 X 125mg and 1 X 250mg per dose. This is to allow the dose to be reduced by 125 mg in each instance, ie to the previous dose, if the patient does not tolerate an increase in dose of study drug or placebo.

Study procedures: Screening procedures consist of a full medical history, full physical, neurological and ophthalmic examination, blood and urine tests and psychometric tests

(ADAS-Cog, MMSE) to confirm the patient's eligibility for the study. Nerve conduction tests and visual evoked responses are conducted between the screening and baseline visits to provide a baseline measurement, and to exclude patients with undiagnosed peripheral neuropathies or visual disturbances. Blood is collected for ApoE allotyping and determination of baseline plasma levels of the compound of formula I or II, metals and Aβ prior to randomisation.

The study lasts 36 weeks, with 13 visits (including screening). Eligible subjects we randomised to receive either the compound of formula I or II or placebo. All patients continue their study entry dose of donepezil, and all patients received 100 µg vitamin B12 IM every four weeks.

***Outcome measures*:** The primary efficacy variable is a change in the baseline score on the Alzheimer's Disease Assessment Scale (ADAS), which is conducted at baseline and at weeks 4, 12, 24 and 36. This readout is chosen for comparability of treatment effects with current therapeutic agents, such as donepezil, for which efficacy trials also used ADAS as their primary outcome measure (Rogers et al., 1998). Although numerous neuropsychological tests could be considered as secondary measures, it is necessary to avoid fatiguing the subjects at review. Therefore the only other cognitive test performed was the Mini-Mental State Exam (MMSE), which is well characterized and easily implemented. The Clinician's Interview Based Impression of Change (CIBIC), a subjective observational index also used in efficacy trials of acetylcholinesterase inhibitors, is conducted at baseline and at weeks 12, 24 and 36 by an independent researcher who was not part of the study team. Blood samples for measurement of plasma Aβ and plasma zinc and copper are all taken 4 weekly, by antecubital fossa venepuncture.

Therapeutic drug monitoring: the compound of formula I or II drug assays are conducted over 6 hours at weeks 12, 24 and 36. The patient's blood is obtained via a heparinized indwelling catheter before the administration of the compound of formula I or II on these days, and then drawn again at 2, 4 and 6 hours post dose. This is done to achieve pharmacokinetic data to correlate with other outcome measures.

Safety measures: Standard adverse event reporting to a safety monitoring committee, consisting of physicians independent of the study, is conducted to review adverse events at three monthly intervals and on an emergent basis. Following baseline, safety visits are conducted at weeks 2, 4, 8, 12, 16, 20, 24, 26, 28, 32 and 36. The patient and carer are questioned about any changes which might have occurred in the patient's

health or medications since the last visit. Standard biochemical, renal and liver function, full blood examination, serum vitamin B12 and folate levels, blood pressure and weight are documented at each visit. A neurological examination is conducted at each visit to assess for peripheral neuropathy and optic neuropathy, and visual evoked responses, nerve conduction studies and a full ophthalmic examination (visual acuity, colour vision, fundal examination and visual field) are conducted at screening, at week 18 and at 2 weeks after trial completion. An ECG was performed at baseline and at weeks 12 and 24.

Extension study: All patients who completed the Phase II trial are invited to continue on a 48 week, prospective, open-label study of the compound of formula I or II. All are allocated to receive the compound of formula I or II 125mg bid, increased after 2 weeks to 250mg bid, then 375mg BD at 4 weeks, while remaining on donepezil and vitamin B12. Patients who failed to tolerate dose increases beyond the 250mg/day, 500mg/day and 750mg/day dosages in the blinded phase are placed on the highest tolerable dose beyond the previously achieved dose, at the clinical discretion of the investigator in the extension phase. Outcome and safety measures are the same as for the blinded phase. The length of the extension study is based upon an estimation of the time required to complete the blinded Phase II clinical trial, so that subjects will be able to continue to take the drug until they could be advised about the results of the trial.

Data preparation and statistical analysis: Concealed randomization is conducted in blocks of 6 by the Institute of Drug Technology, a body which is independent of the study. An independent data monitoring company checked for omissions and validated entries in case report forms, and double entered the data into Microsoft Access©, completing validation and consistency checks. Before the analyses, each patient's randomization arm is labelled either 'A' or 'B'. This ensures that the primary analyses are conducted blind to the subjects' randomization group, and hence were triple blind).

Two-way analysis of variance and covariance is used to analyse the major outcome variables with group (treatment vs placebo) as a between-subjects factor and occasion (baseline vs subsequent measurement occasions) as a within-subjects factor. Evidence for efficacy is indicated by a significant group by occasion interaction. Differences between groups on categorical measures are analysed using exact statistical methods in order to maximise power.

The influence of confounding variables is controlled using analysis of covariance and linear regression models where appropriate. Based on the assumption of 50% shared variance between measurement occasions (i.e. r=0.70), power to detect

an effect of one standard deviation difference in change between groups from baseline to week 36 would be approximately 80% if 15 subjects were recruited per group. Since an attrition rate of 15% has been observed in similar populations, 18 patients will be recruited into each arm.

The design also includes a subset analysis of outcome measures, in which the cohort is divided into two equal size groups by the median ADAS-Cog score at baseline, yielding a less severely-affected subset, and a more severely-affected subset.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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